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Decoding checkpoint inhibitor-induced immune-related adverse events, volume II

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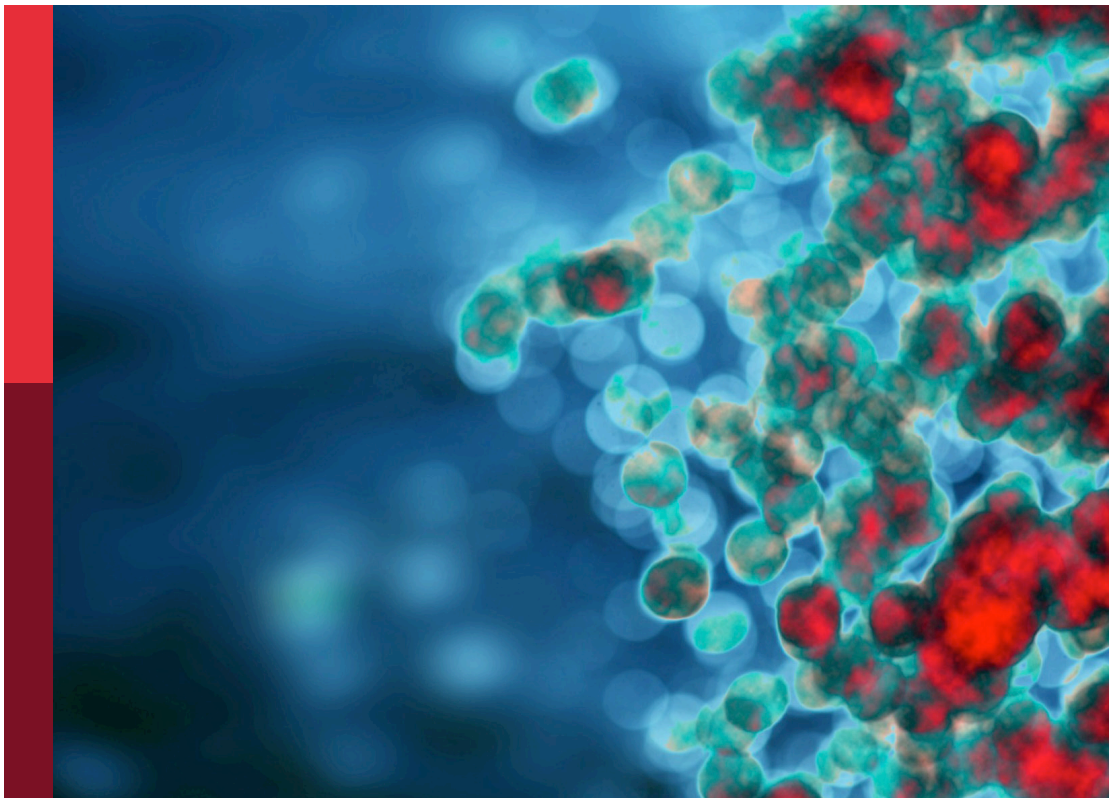

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Decoding checkpoint inhibitor-induced immune-related adverse events, volume II

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Editorial: Decoding checkpoint inhibitor-induced immune-related adverse events, volume II

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Editorial on the Research Topic
Decoding checkpoint inhibitor-induced immune-related adverse events, volume II

Immune checkpoint inhibitors (ICIs), particularly those targeting the Programmed Death 1 (PD-1) or Cytotoxic T-Lymphocyte Associated 4 (CTLA-4) axes have redefined cancer treatment outcomes. The capacity of these therapies to redirect the immune system is linked to off-target toxicities. These immune-related adverse events (irAEs) exhibit varied clinicopathological features. This Research Topic builds on our previous topic focusing specifically on ICI-induced Endocrinopathies (1) and extends into a wider range of immune toxicities. This Research Topic incorporates disciplines including endocrinology, immunology, oncology, gastroenterology, diagnostic radiography and epidemiology to help guide clinical practice and research directions.

The topic is framed through a bibliometric analysis by Jiang et al. which charts the field's evolution over 2005-2022. Initially slow, interest in the area surged from 2015. irAE papers were published in both cancer and immunology journals, utilising a variety of keywords, highlighting a challenge for researchers seeking information on the topic.

Anpalakhan et al. presented a sub-analysis of the Spinnaker study, a retrospective multicentre observational study exploring patients with non-small-cell lung cancer receiving pembrolizumab with platinum-based chemotherapy (2). The study reported that 43% experienced irAE: two-thirds Grade 1–2, one-third Grade 3–4. The study

replicated what has been observed in other studies: that the development of irAEs portends a better intervention response. Further research in immune markers of irAEs for early intervention and response is required.

This Research Topic also delves, through the work of Zhao et al., to focus on pancreatitis; a rare but significant irAE. The team analysed over 40,000 patients in 59 randomized controlled trials (RCTs), reporting an incidence of pancreatitis of 0.93% for single-agent ICI, and 1.1% for combination blockade. The group found patients treated with immunotherapy for melanoma had the highest incidence of amylase/lipase elevation, and that pancreatitis was more common with PD-1 compared with PD-L1 blockade. This review adds relevant insight to understanding which populations might benefit from proactive pancreatitis monitoring.

The latest evidence on PD-1 inhibitor-associated gastrointestinal toxicity is reviewed by Cheng et al., including clinical manifestations, grading, mechanisms, treatments, biomarkers, and risk-stratification. This comprehensive review raises the importance of PD-1 rechallenge after ICI-related colitis, which can lead to colonic perforation. The review highlights the need for further research on coliits indicators to clarify the risk benefit balance in this setting.

This Research Topic also incorporates studies focusing on improved strategies for monitoring and predicting irAEs.

Baier et al. describe PD-L1 upregulation in intra-renal and urinary kidney cells as a pathology biomarker. They propose evaluation of urinary PD-L1-positive kidney cells for biomonitoring for ICI-related nephrotoxicity. The study sets precedent for exploring non-conventional (but clinically-feasible) monitoring techniques to improve diagnosis and irAE outcomes.

Huang et al. reviewed specific features of ICI-related pneumonitis by computed tomography (CT). They emphasise the potential of CT radiomics to distinguish ICI-related pneumonitis (CIP) compared to pneumonitis induced by radiation. This review highlights the high-accuracy of radiomics models to predict the development of CIP from CT images and the potential these tools hold for pneumonitis management. Huang et al. noted the challenges of machine standardisation and stress the need for larger comparative studies in the field.

Advances in theranostics facilitate identification of tissue infiltrating CD8+ T-cells using PET imaging. Bol et al. present a melanoma patient who developed ICI-related hypophysitis. Imaging detected increased pituitary CD8+ infiltration, with concomitant tracer uptake in known cerebral metastasises, indicating ICI-induced CD8+ tumor infiltration. These findings support the role of CD8+ T-cells in the tumouricidal effects of ICI, and their role in irAEs, although further validation from clinical trials is required.

Zhang et al. sourced irAE reports from the US Food and Drug Administration's Adverse Event Reporting System to establish improved predictive irAE models. They linked these reports with data across 22 cancer types, uncovering key factors linked to irAE frequency. These factors included the tumour mutational burden (TMB), immune composition and expression signatures and transcriptional expression of checkpoint molecules. This study built composite models combining TMB, naïve CD4+ T cells and dendritic cells which displayed high predictive accuracy for irAE occurrences. Zhang et al. also utilised mRNA expression to develop gene-based predictive models. Interestingly, many of the factors they identified lack known associations with ICI efficacy, underscoring the significance of irAE-specific correlation models.

This Research Topic also incorporated studies exploring irAEs from less well-established ICI therapies. This includes combination regimes involving taxane-based chemotherapeutic nanoparticles, such as nab-paclitaxel (nab-PTX) and PTX. Hao et al. conducted a meta-analysis, encompassing 22 published RCTs with 15962 patients, to assess the risk of irAEs when comparing ICI monotherapy to combination therapy with nab-PTX/PTX. Their findings suggested that combination therapy reduced the risk of specific irAEs, particularly those related to thyroid dysfunction or pneumonitis. However, the impact of combination therapy on other irAEs was less conclusive. The underlying mechanism and potential confounding factors, such as corticosteroid pre-treatment in PTX chemotherapy, warrant further investigation.

The topic also serves as a focus point for unusual presentations of irAEs.

This includes a case report by Huo et al. describing severe grade thyrotoxicosis following treatment of hepatocellular carcinoma treated with chemotherapy combined with the PD-1 inhibitor tislelizumab. Symptomatic thyrotoxicosis due to thyroidits occurred after two cycles of combined treatment, complicated by rapid atrial fibrillation, resulting in dose interruption, and requring treatment with antihistamine, methimazole, and methylprednisolone. The patient became hypothyroid four-months post-thyrotoxicosis. This case is notable considering that most thyroid irAE previously reported are generally mild. Currently, there are no predictors for severe thyrotoxicosis, though there are potential candidates include pre-existing autoimmunity and thyroid autoantibodies (3).

Li et al. have presented a small case series reported PD-1-associated urethritis and cystitis, perhaps less commonly recognised than the more common nephritis. The series highlights the benefit of a detailed history for symptoms of genitourinary inflammation, simple screening to discern this from a urinary tract infection, with the benefit of avoiding unnecessary antibiotic therapy.

Together this Research Topic serves as an important contribution to the field of ICI-induced irAEs. It provides a comprehensive summary of the field to date through a bibliometric report of the topic and a deeper analysis of pancreatitis and gastrointestinal toxicity irAEs. The topic explores strategies for improved monitoring and prediction of irAEs. These include upregulated PDL-1 intra-renal and urinary kidney cells, CT radiomics, T-cell distribution and a variety of models using RNA, cellular and tumour immune correlates. This Research Topic also describes the incidence of irAEs from less well-established ICI therapies and reports of unusual irAE occurrences from standard ICI

treatments. Together this diverse Research Topic synergistically melds many areas of the field in a single focus point resource.

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Immunogenomic correlates of immune-related adverse events for anti–programmed cell death 1 therapy

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Despite impressive antitumor efficacy of programmed cell death 1 (PD-1) inhibitors, this inhibition can induce mild to severe autoimmune toxicities, termed immune-related adverse events (irAEs). Yet, predictive pretreatment biomarkers for irAEs development across cancer types remain elusive. We first assessed cellular and molecular factors. To determine factors predicting the risk of irAEs for anti–PD-1 immunotherapy across multiple cancer types, an integrative analysis of cellular and molecular factors from 9104 patients across 21 cancer types and 4865522 postmarketing adverse event reports retrieved from adverse event reporting system was then performed. Accuracy of predictions was quantified as Pearson correlation coefficient determined using leave-one-out cross-validation. Independent validation sets included small cell lung cancer and melanoma cohorts. Out of 4865522 eligible adverse events reports, 10412 cases received anti–PD-1 monotherapy, of which, 2997 (28.78%) exhibited at least one irAE. Among established immunogenomic factors, dendritic cells (DC) abundance showed the strongest correlation with irAEs risk, followed by tumor mutational burden (TMB). Further predictive accuracy was achieved by DC and TMB in combination with $CD4^+$ naive T-cells abundance, and then validated in the small cell lung cancer cohort. Additionally, global screening of multiomics data identified 11 novel predictors of irAEs. Of these, *IRF4* showed the highest correlation. Best predictive performance was observed in the *IRF4* – *TCL1A* – SHC-pY317 trivariate model. Associations of *IRF4* and *TCL1A* expression with irAEs development were verified in the melanoma cohort receiving immune checkpoint inhibitors. Collectively, pretreatment cellular and molecular irAEs-associated features as well as their combinations are identified regardless of cancer types. These findings may deepen our knowledge of irAEs pathogenesis and, ultimately, aid in early detection of high-risk patients and management of irAEs.

KEYWORDS

immune-related adverse event, cellular biomarker, molecular biomarker, immune cell, immunotherapy

Introduction

Immune checkpoint inhibitors (ICIs) targeting programmed cell death 1 (PD-1) pathway have brought remarkable clinical benefits in diverse cancers (1). The ICIs work by blocking the PD-1 from binding with its partner proteins, resulting in immune activation in the tumor microenvironment (2). Nevertheless, ICI use is commonly associated with autoimmune toxicities, known as immune-related adverse events (irAEs) (3). The most common irAEs are observed in skin, colon, endocrine organs, liver, and lungs, but any organ can be affected and some infrequent irAEs may be serious and fatal, such as encephalitis and myocarditis (3, 4). Pre-existing autoantibodies (5), gut microbiome (6), tissue-resident T-cells (7), microRNAs (8), and cytokines (6) have all been involved in irAEs in single cancer type. The pathogenesis of irAEs remains poorly characterized and no biomarkers are routinely used in standard clinical practice to recognize patients at high risk for irAEs development.

Although high tumor mutational burden (TMB) has recently been reported to correlate with elevated irAEs risk across cancer types (9), large proportion (> 50%) of variation in irAEs risk has not yet been accounted for during anti–PD-1 therapy, indicating the role of other factors in leading to irAEs. Herein we systematically study this hypothesis, aiming to identify additional pretreatment immunogenomic factors that contribute to irAEs development regardless of cancer types. To this end, we analyzed cleaned large-scale pharmacovigilance data of irAEs from The US Food and Drug Administration's Adverse Event Reporting System (FAERS) and The Cancer Genome Atlas (TCGA) multiomics data from whole-exome sequencing, mRNA sequencing, microRNA sequencing, and reverse phase protein array across multiple cancer types, and lastly, validated our hypothesis in independent cohorts.

Materials and methods

Details about the methods are provided in Supplementary Methods, and a flow chart illustrating main analyses conducted is presented as Supplementary Figure S1.

IrAEs risk evaluation

FAERS is a database of spontaneously gathered adverse event reports, containing great collection of reports of irAEs on anti–PD-1 agents in a real-world situation. A FAERS search engine named OpenVigil (version 2.1) was used to retrieve cleaned adverse event reports (10). Only reports with nivolumab or pembrolizumab as the suspected cause of adverse events were considered. Further, given that overall prevalence of irAEs and severity was higher with combined PD-1 and cytotoxic T-lymphocyte associated protein 4 (CTLA-4) antibodies as compared with monotherapy (11, 12), anti–PD-1 agents plus ipilimumab combination therapy was excluded. The irAEs reported in FAERS were defined as 106 preferred terms in the Medical Dictionary for Regulatory Activities according to previously published irAEs management guidelines during ICI therapy (3, 4, 13, 14), and listed in Supplementary Table S1. Lastly, reporting odds ratio (ROR) was calculated for each cancer type to evaluate the risk of a cancer type developing any irAE, which represents standard practice for quantitative analyses of data in spontaneous reporting systems such as FAERS (9, 15, 16).

Molecular and cellular data sources from TCGA

Datasets of somatic mutations, mRNA, microRNA, and protein expression for 9104 samples across 21 cancer types (Supplementary Figure S1) with available irAEs ROR data were downloaded from the TCGA Pan–Cancer Atlas project hosted in the UCSC Xena Hubs (17). TMB was then calculated as the count of nonsynonymous mutations for each patient based on somatic mutations, and log-transformed. On the basis of the mRNA expression dataset, several tumor immune microenvironment-related signatures were generated, including cytolytic index to assess intratumoral cytolytic activity (18), interferon (IFN)-gamma and expanded immune signatures (19), and a transcriptional signature reflecting $CD8^+$ T-cells exhaustion (20). Proportion of PD-1-high samples for each cancer type was also determined, with percentile 80^{th} of PD-1 mRNA expression in the entire TCGA cohort as the cutoff (21).

Other immunogenomic factors, including T cell receptor (TCR) diversity, intratumor heterogeneity, and neoantigen load, were obtained from Genomic Data Commons Pan-Cancer Atlas panimmune data portal (22).

Abundance data of 30 immune cell types in the tumor microenvironment for the TCGA samples were inferred using xCell (23) and downloaded from the xCell website. The abundance was defined as an enrichment score which showed resemblance to the fraction of specific cell type in the tumor microenvironment (23).

Lastly, median values of individual aforementioned immunogenomic factors except the PD-1-high samples proportion were calculated for each cancer type. Raw data of mRNAs, microRNAs, proteins, and phosphoproteins were preprocessed separately, and then their median expression levels per cancer type were determined for further analyses.

Objective response rates across cancer types

Objective response rate (ORR) for PD-1 or its ligand PD-L1 inhibitor monotherapy across 19 types of cancers

(Supplementary Figure S1) was compiled from Yarchoan et al. (24). To evaluate the correlation of tumor response with irAEs risk, Pearson correlation coefficient (*R*) between the ORR and the corresponding irAEs ROR across these cancer types was calculated.

Small cell lung cancer and melanoma cohorts

Given that molecular data for small cell lung cancer is lacking in TCGA but its irAEs ROR could be calculated in our study, we focused on an independent cohort encompassing 71 small cell lung cancer patients with both somatic mutations and mRNA sequencing data of pretreatment tumors available (25). TMB was calculated as previously described. To estimate abundances of immune cells, gene expression data in fragments per kilobase of exon per million reads mapped units was fed to the R package xCell (23). The ICIs-treated cohort in our study consisted of 60 patients with metastatic melanoma, which received either anti–PD-1 blockade (nivolumab or pembrolizumab) or nivolumab plus ipilimumab therapy (26). All irAEs were classified according to the United States Health and Human Services Common Terminology Criteria for Adverse Events v.5.0. Grade 0 reflected no toxicity, and irAEs occurrence was defined as grade 1+. RNA sequencing was performed for peripheral blood mononuclear cell samples obtained at baseline, and then read counts were normalized to gene-level transcripts per million (TPM) for further differential gene expression analyses against irAEs status.

Statistical analysis

To examine the relationships of molecular and cellular factors with irAEs risk, Pearson correlation analysis was used to calculate the *R*s between their respective medians and the ROR across the 21 cancer types above. For combinations of irAEs risk-associated factors, a multivariable linear regression analysis with leave-one-out cross-validation in predicting ROR across cancer types was performed using the R package caret. Prediction performance of linear models was determined as *R* and unexplained variance ($1 - R^2$). Multicollinearity among variables in a multivariable linear model was quantified as variance inflation factor (VIF) calculated using the R package rms; a VIF > 4 was considered as an indicator of multicollinearity. Log-likelihood ratio test was applied to comparing the goodness-of-fit between different models using the R package lmtest. Specifically, the log-likelihood ratio test was conducted between the bivariate model and corresponding single variable models to determine the bivariate model fitness; for the trivariate model fitness comparison, the log-likelihood ratio test was conducted between the trivariate model and corresponding bivariate models. Multiple testing correction was performed to control the false discovery rate (FDR) by the Benjamini-Hochberg method. All *P* values were 2-sided and statistical significance was expected at FDR <.05 unless stated otherwise.

In the melanoma cohort, Mann-Whitney *U* test was used to compare the difference in gene expression between irAEs status. To eliminate the possibility that the associaton between gene expression and irAEs status was skewed by ICIs therapy type, a logistic regression model was adopted to control for different therapy classes. All statistical analyses were done in R statistical software v.3.5.2.

Results

Association of established immunogenomic factors with irAEs risk

A total of 4865522 reports were identified in FAERS, including 10412 cases that received the anti–PD-1 monotherapy for 22 cancer types. Of those 10412, 2997 cases (28.78%) exhibited at least one irAE. As shown in Supplementary Figure S2, the irAEs RORs varied between cancer types, ranging from the lowest 0.94 in cholangiocarcinoma to the highest 5.87 in melanoma.

Given that the relationship between irAEs onset and survival advantage of patients on ICIs has been shown in large case series studies in multiple cancers (27), we first examined the correlation of irAEs ROR with ORR. Our analysis demonstrated a significant positive correlation between them ($R = 0.51$; $P = .03$) (Supplementary Figure S3). Next, we investigated whether established immunogenomic correlates of response to ICI therapy may associate with irAEs risk. Strong association signals were identified for 15 factors (P <.05 for all), with 12 passing the correction for multiple testing (FDR <.05 for all): 5 immune cells, TMB, 3 immune expression signatures, and 3 checkpoint-related factors. (Figure 1A). Specifically, the strongest correlation between dendritic cells (DC) abundance and ROR was observed ($R = 0.69$; FDR = .02) (Figure 1B), suggesting that 48% of the differences in ROR across cancer types can be explained by DC. Estimated abundances of all other immune cell types were not significantly correlated with irAEs risk, except for $CD8^+$ T-cells ($R = 0.57$; FDR = .04), Mast cells ($R = 0.57$; FDR = .04), $CD4^+$ T-cells ($R = 0.56$; FDR = .04), and $CD4^+$ naive T-cells ($R = 0.55$; FDR = .04) (Figure 1A, Supplementary Figures S4A–D). Consistent with the previous study (9), elevated TMB was demonstrated to correlate with increased risk of irAEs ($R = 0.63$; FDR = .04) (Figure 1C). Additionally, 3 immune expression signatures — expanded immune signature, IFN-gamma signature, and cytolytic index — which are related to IFN-gamma signaling and activated T cell biology (18, 19), displayed significant correlations with ROR (Supplementary Figures S4E–G). We also found that checkpoint-related factors, including individual transcriptional expressions of

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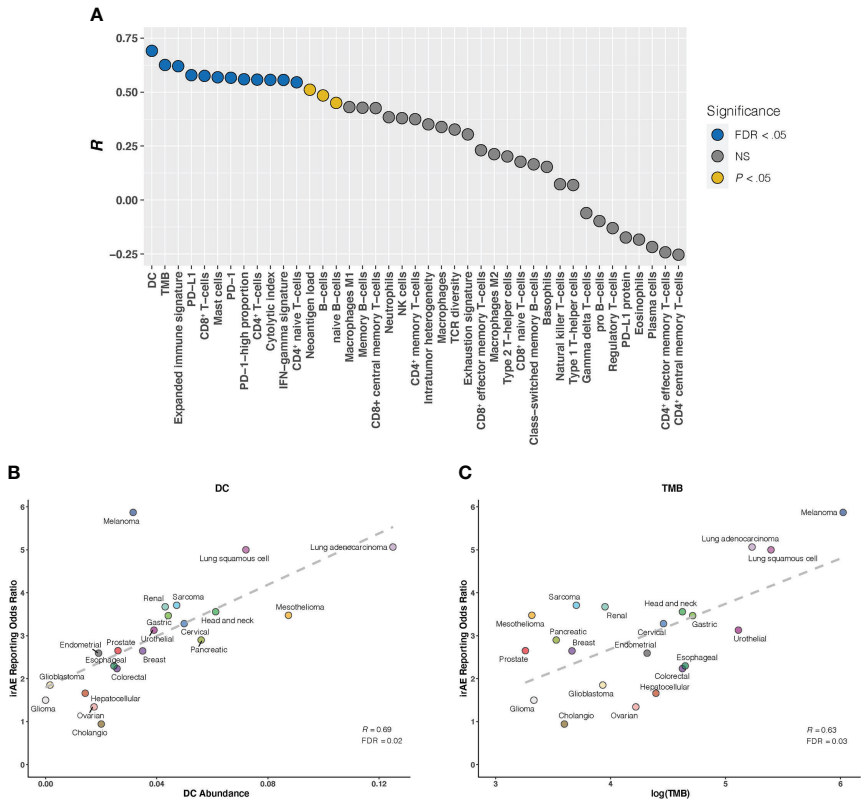

FIGURE 1
Correlation Between Established Immunogenomic Factors and Immune-Related Adverse Events for Anti–PD-1 Therapy Across Cancer Types **(A)**, Evaluation of immunogenomic correlates of immune-related adverse events (irAEs) for immunotherapy to block the PD-1 pathway across cancer types. The horizontal axis denotes the 41 established immunogenomic factors, and the vertical axis denotes Pearson correlation coefficient (*R*). Circles are filled with distinct colors as per statistical significance of corresponding factors. **(B, C)**, Correlation of dendritic cells abundance (DC) **(B)** and log(tumor mutational burden [TMB]) **(C)** with the reporting odds ratio of any irAE across 21 cancer types which are color coded. The dashed line depicts the linear fit. FDR, false discovery rate; NS, not significant.

PD-L1 and PD-1, and PD-1-high-proportion, may contribute to irAEs onset (Supplementary Figures S4H–J).

Combination of DC, TMB, and CD4$^+$ naive T-cells for irAEs risk prediction

We further investigated whether certain combinations of those 12 correlates of irAEs ROR could provide additional accuracy in predicting irAEs risk. The performances of 66 bivariate models were first evaluated. Of these 31 models showed significant statistical differences compared with their respective univariate models in terms of the fitness (log-likelihood ratio test, P <.05 for all) and no signs of collinearity were detected (VIF < 4 for all) (Figure 2A; Supplementary Table S2). However, only TMB and CD4$^+$ naive T-cells or DC bivariate models outperformed the DC-based univariate model (TMB – CD4$^+$ naive T-cells model, R = 0.73; TMB – DC model, R = 0.71; both FDR = .01) (Figures 2B, C). Of note, some cancer types, which had RORs higher than would be predicted by the TMB – CD4$^+$ naive T-cells model, exhibited higher DC abundance (e.g., lung adenocarcinoma), and some with lower-than-predicted RORs showed lower DC abundance (e.g., glioma) (Figure 2B). The same was true for CD4$^+$ naive T-cells abundance in the TMB – DC bivariate model (Figure 2C). Thus, we next examined whether inclusion of the third variable would aid in contributing additional information beyond the bivariate model. Indeed, of the resulting trivariate models, combined DC, TMB, and CD4$^+$ naive T-cells model achieved the best predictive accuracy (R = 0.81; FDR = 1.1×10^{-4}), and exhibited pronounced model promotion in comparison with their corresponding bivariate models (log-likelihood ratio test, P = 8.7×10^{-4} relative to TMB – DC model; P = 2.8×10^{-4} relative to TMB – CD4$^+$ naive T-cells model) (Figure 3; Supplementary Table S3). Likewise, there was no sign of collinearity for this trivariate model (Supplementary Table S3). Collectively, these

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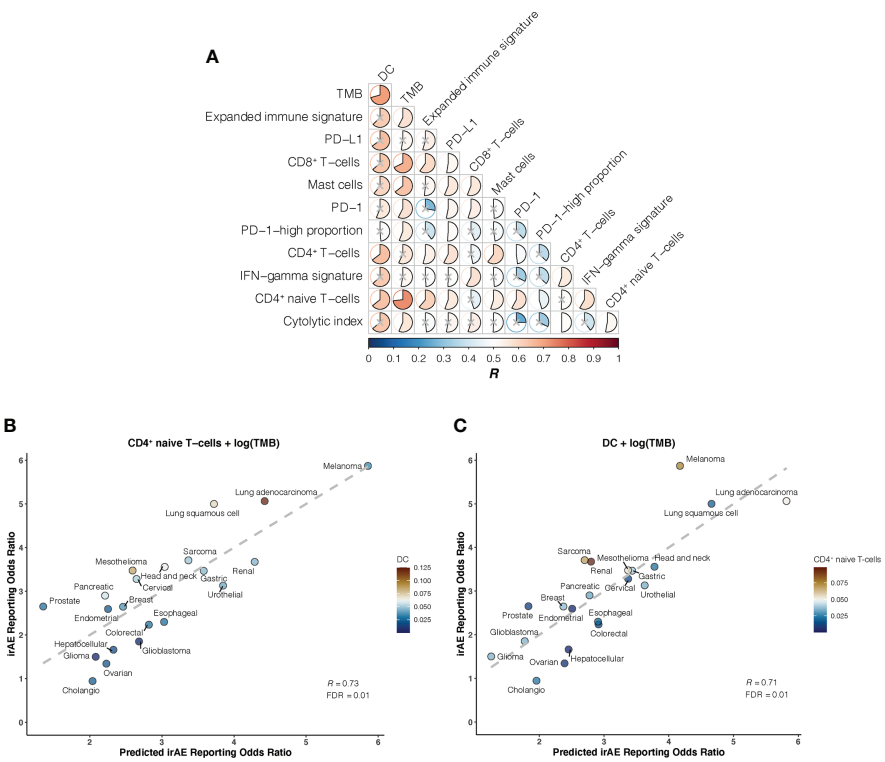

FIGURE 2
Bivariate Models of Candidate Immunogenomic Factors for Predicting Immune-Related Adverse Events for Anti–PD-1 Therapy Across Cancer Types **(A)**, Graph shows performance of bivariate models in predicting immune-related adverse events (irAEs) risk for combinations of the 12 candidate immunogenomic factors. Pearson correlation coefficient (*R*) is represented in colors from dark blue to dark red as shown in the color bar. Color intensity and the size of each pie is proportional to the correlation coefficient. A lack of statistical significance by log-likelihood ratio test (*P* >.05) is indicated with a gray cross. **(B)**, Combined effect of $CD4^+$ naive T-cells abundance (naiveCD4T) and tumor mutational burden (TMB) bivariate model. The dashed line depicts the linear fit, with the formula reporting odds ratio (ROR) = 24.41 × naiveCD4T + 1.01 × log(TMB) − 2.09. The dendritic cells (DC) abundance of each cancer type is color coded where blue indicates low abundance and red, high abundance. **(C)**, Combined effect of DC and TMB bivariate model. The dashed line depicts the linear fit, with the formula ROR = 24.37 × DC + 0.8 × log(TMB) − 1.41. The $CD4^+$ naive T-cells abundance of each cancer type is color coded where blue indicates low abundance and red, high abundance.

results emphasized the importance of integrating multiple factors in determining irAEs risk.

External validation of DC – TMB – $CD4^+$ naive T-cells model

Having identified candidate composite models of irAEs risk, we next attempted to verify our findings in an independent cohort of small cell lung cancer, a cancer type not depicted in TCGA and known to have high TMB but low response rates to ICIs. As shown in Figure 3B, estimated ROR by univariate TMB model markedly deviated from the actual ROR of 2.71. This striking deviation was also observed in previous work showing substantially lower-than-anticipated ROR for small cell lung cancer (9). However, strong improvements were seen after incorporating DC and/or $CD4^+$ naive T-cells into our prediction models, further supporting the validity of synergistic combination of DC, TMB, and $CD4^+$ naive T-cells.

Dissection of novel molecular predictors for irAEs risk

As a further step toward understanding irAEs development and identifying novel molecular determinants not reported to be implicated in ICI response, thus boosting irAEs risk prediction, we correlated large-scale expression profiling data for mRNA, microRNA, and protein with irAEs ROR across 21 cancer types. 11 significant predictors of irAEs risk were identified (Figures 4A, B; Supplementary Figures S5-7), such as mRNA expressions of *IRF4* (OMIM 601900), *TCL1A* (OMIM 186960), *GPNMB* (OMIM 604368), and *FAIM3* (OMIM 606015). Of these, the transcription factor *IRF4* showed the highest correlation with ROR (*R* = 0.847; FDR = .02) (Figure 4A), possibly relating to its essential roles in many aspects of B-cells, T-cells, and DC differentiation and function (28–31). The next highest correlation was observed for *TCL1A* (*R* = 0.82; FDR = .04) (Figure 4B), which is a critical player in several lymphoid malignances, and has been demonstrated to act as a coactivator to augment the activity of AKT kinases (32),

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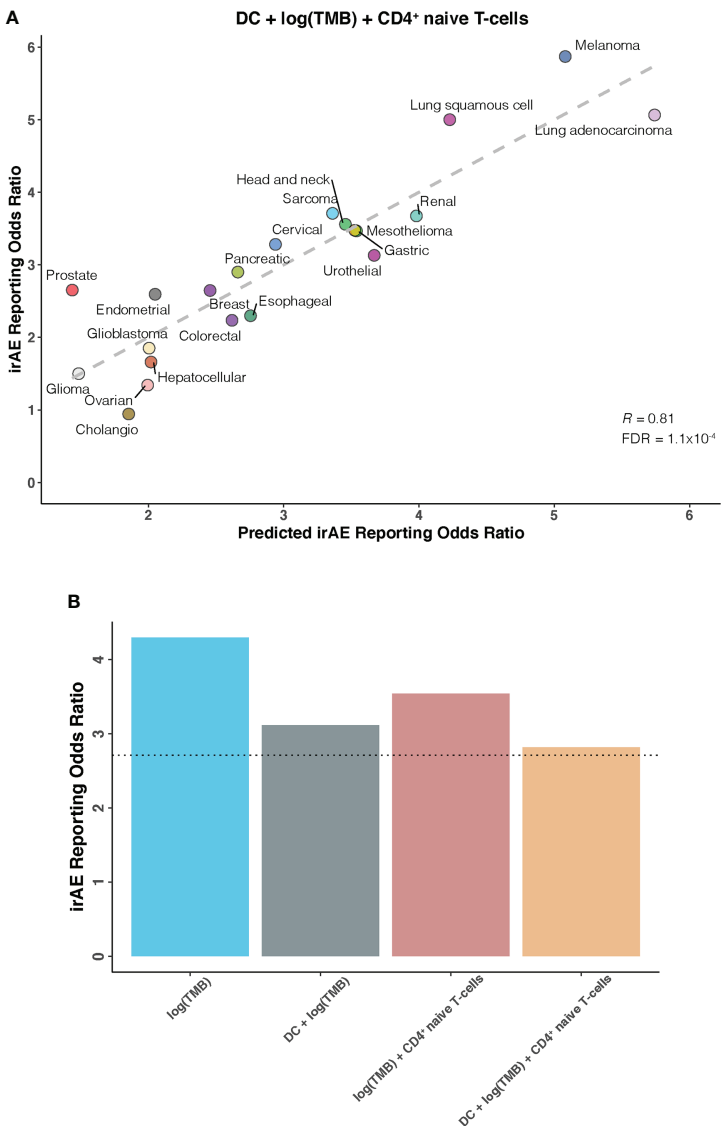

FIGURE 3
DC – TMB – $CD4^+$ Naive T-cells Trivariate Model for Predicting Immune-Related Adverse Events for Anti–PD-1 Therapy Across Cancer Types **(A)**, Combined effect of dendritic cells abundance (DC) – tumor mutational burden (TMB) – $CD4^+$ naive T-cells abundance (naiveCD4T) trivariate model. The dashed line depicts the linear fit, with the formula reporting odds ratio (ROR) = 19.03 × DC + 0.82 × log(TMB) + 18.03 × naiveCD4T – 1.85. **(B)**, Estimated ROR to anti-PD-1 therapy in small cell lung cancer based on TMB univariate model, DC – TMB and TMB – $CD4^+$ naive T-cells bivariate models, and DC – TMB – $CD4^+$ naive T-cells trivariate model. The dotted line represents the ROR in small cell lung cancer, which was obtained using The US Food and Drug Administration's Adverse Event Reporting System.

thus serving as a downstream effector of B-cell receptor and TCR-mediated signaling (33). Interestingly, two additional genes, *GPNMB* (also known as *DC-HIL*) and *FAIM3*, showing positive associations with ROR (R = 0.81; FDR = .049) (Supplementary Figure S5), also had well-described roles in regulating immunity (34–37). We also noted that SHC phosphorylation level on Tyr317 (SHC-pY317) was negatively correlated with ROR (R = -0.75; FDR = .02) (Supplementary Figure S6). A study in mice indeed identified that deficiency of p66Shc protein, a homolog of human gene *SHC*, resulted in negative regulation of lymphocyte activation and autoimmunity (38). Other hits included 6 positively associated microRNAs such as miR-155-3p (R = 0.73; FDR = .02) (Supplementary Figure S7). Strikingly, miR-155 has emerged as a multifaceted mediator of innate and adaptive immunity and may drive, when deregulated, aberrant immune responses, such as the development of autoimmune disorders (39, 40).

Of bivariate models derived from aforementioned correlates (Supplementary Figure S8; Table S4), the *IRF4* – *TCL1A* model yielded optimal prediction accuracy (R = 0.854; FDR = 2.3×10^{-5}) (Figure 4C). Although the increment of R was small compared

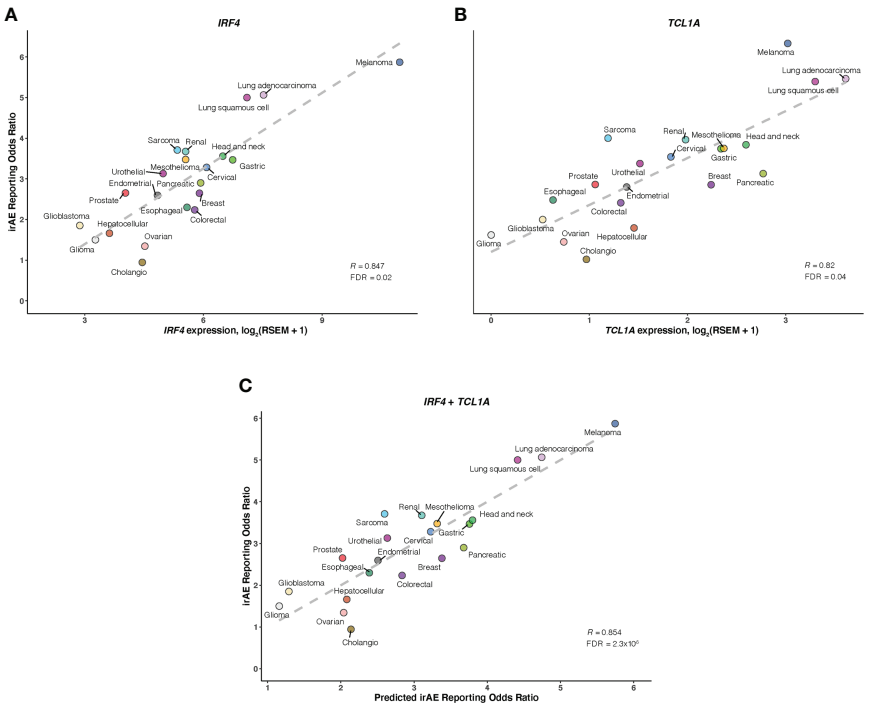

FIGURE 4
Association of *IRF4* and *TCL1A* with Immune-Related Adverse Events for Anti–PD-1 Therapy Across Cancer Types A and B, Correlation of *IRF4* **(A)** and *TCL1A* **(B)** mRNA expression with the reporting odds ratio (ROR) of any immune-related adverse event (irAE) across 21 cancer types which are color coded. The dashed line depicts the linear fit. **(C)**, Combined effect of *IRF4* and *TCL1A* expression bivariate model. The dashed line depicts linear fit, with the formula ROR = 0.38 × IRF4 + 0.54 × TCL1A − 0.1.

with that from *IRF4* alone, the log-likelihood ratio test indicated a significant model improvement ($P = .02$ relative to *IRF4* model; $P = 3\times10^{-3}$ relative to *TCL1A* model). We then incorporated each of 9 other factors into the *IRF4* – *TCL1A* bivariate model successively, and found significant enhancement on the prediction performance only in the *IRF4* – *TCL1A* – SHC-pY317 trivariate vs *IRF4* – *TCL1A* bivariate models ($R = 0.87$; FDR = 2.5×10^{-6}; log-likelihood ratio test, $P = .03$) (Figures 5A, B; Supplementary Table S5).

Given these results, we asked whether a composite model integrating the 11 novel molecular determinants with the 12 prior immunogenomic ones could outperform the *IRF4* – *TCL1A* – SHC-pY317 model. In contrast, none of constructed bivariate models outperformed it (Supplementary Table S6). Moreover, the combination of DC, TMB, $CD4^+$ naive T-cells, *IRF4*, *TCL1A*, and SHC-pY317 did not improve our ability to predict irAEs risk ($R = 0.81$).

Validation of IRF4 and TCL1A associated with irAEs in ICI-treated melanoma cohort

Lastly, we examined associations of *IRF4* and *TCL1A* genes with irAEs development in patients with melanoma receiving ICI treatment. As shown in Figure 6A, *IRF4* mRNA level was significantly elevated in patients developing irAEs compared with those without any irAEs (median expression, 4.36 vs 3.98; Mann-Whitney *U* test, $P = .04$). We next wondered whether this association was skewed by ICI therapy type. After correcting for therapy classes, *IRF4* remained associated with irAEs development (logistic regression, $P = .03$). In contrast, the difference in *TCL1A* mRNA level stratified by irAEs status was not statistically significant, although there was a trend toward high *TCL1A* expression in irAEs-experiencing patients subgroup (median expression was 1.32 for irAEs-experiencing patients vs 0.75 for irAEs-free ones; Mann-Whitney *U* test, $P > .05$). Further analysis revealed that patients with grade 3, 1, or no irAEs had higher *TCL1A* expression than those experiencing the most severe irAEs (median expressions for grade 4, 3, 1, and 0 were 4.7, 1.61, 1.32, and 0.81, respectively; Mann-Whitney *U* test, $P < .05$ for all) (Figure 6B).

Discussion

Our integrative analyses of large-scale cleaned pharmacovigilance data and multiomics profile offer a valuable collection of baseline predictors for irAEs development regardless of caner types, as

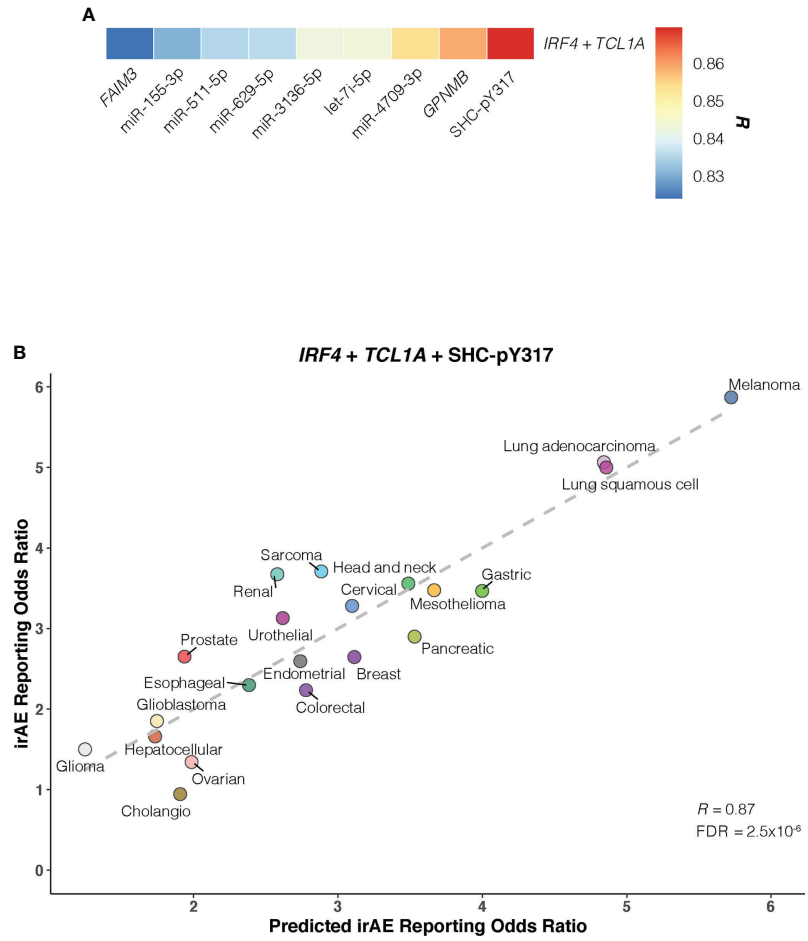

FIGURE 5
Optimal *IRF4 – TCL1A* – SHC-pY317 Model in Predicting Immune-Related Adverse Events for Anti–PD-1 Therapy Across Cancer Types. **(A)**, Comparison of predictive performance given by the *IRF4* and *TCL1A* genes together with other immune-related adverse events (irAEs)-associated molecular factors. Pearson correlation coefficient (*R*) is represented in colors from blue to red as shown in the color bar. **(B)**, Combined effect of *IRF4 – TCL1A* – SHC-pY317 trivariate model. The dashed line depicts the linear fit, with the formula reporting odds ratio = 0.28 × IRF4 + 0.47 × TCL1A – 2.39 × SHC-pY317 + 0.77. Cancer types are depicted in distinct colors.

illustrated by the identified 22 other risk factors beyond TMB. Moreover, proper factor combination can markedly improve the accuracy of irAEs risk prediction, emphasizing the necessity of concurrent consideration of multiple features in assessing irAEs development. Many features identified herein have been implicated in autoimmunity, thus raising the possibility of common immunological mechanisms underlying both irAEs development and autoimmune diseases.

In our work, we first investigated the relationships between the 41 established immunogenomic factors and irAEs risk, then found 12 irAEs-related factros, including 5 immune cells, TMB, 3 immune expression signatures, and 3 checkpoint-related factors. Of these, 4 cell types (DC, Mast cells, $CD4^+$ T-cells, and $CD4^+$ naive T-cells) have not yet been demonstrated to be associated with ICI efficacy. Next, we investigated the relationships between genome-wide mRNA, microRNA, and protein profiles and irAEs risk, and found 11 *de novo* generated irAEs-related molecular factors, all of which were not reported to be implicated in ICI efficacy. Actually, there was only moderate correlation between irAEs risk and ICI efficacy, implying that immunological mechanisms underlying irAEs development and efficacy were not completely shared. Thus we indeed identified some factors predictive of irAEs risk but not efficacy, although further experimental study is warranted to classify the biological significance of novel features identified in our study.

Based on aforementioned predictors, a trivariate model combining DC, TMB, and $CD4^+$ naive T-cells, which considerably reduced the unexplained variance in predicting irAEs risk from 0.60 ($1 - 0.63^2$) utilizing TMB alone to 0.34 ($1 - 0.81^2$), was generated, suggesting that all these factors may get involved in the mediation of irAEs development.

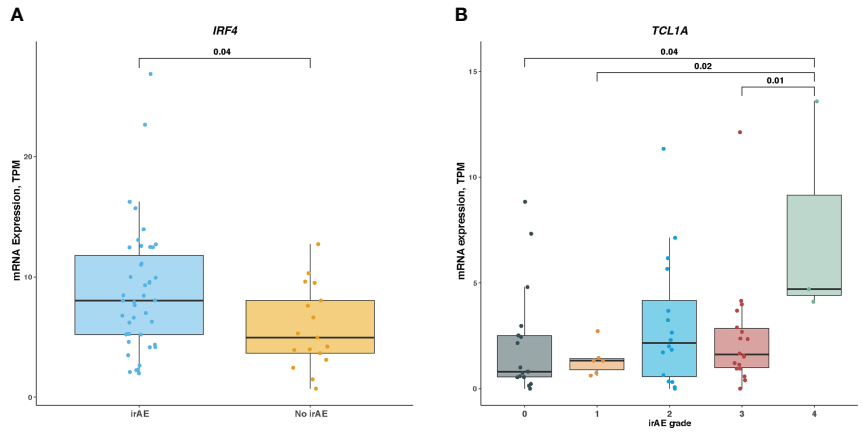

FIGURE 6
Association of *IRF4* and *TCL1A* Expression With Immune-Related Adverse Events in ICI-Treated Melanoma Cohort **(A)**, Comparison of *IRF4* mRNA level in irAEs-experiencing patients vs irAEs-free ones. **(B)**, Comparison of *TCL1A* mRNA level among different irAEs grades. Box plots show the median, first, and third quartiles; error bars extend to 1.5 times the interquartile range; and the dots denote patients profiled by mRNA sequencing. The Mann-Whitney *U* test was used to determine significance. TPM, transcripts per million.

We hypothesized that high TMB may contribute to increased irAEs risk due to consequent increment in immunogenic neoantigens, which could resemble peptides in normal tissues and be recognized as non-self antigens by the immune system (41), thus eliciting irAEs in target tissues as cross-reactive immune responses in the ICI therapy setting. This hypothesis invokes the theory of molecular mimicry that has been involved in the pathogenesis of autoimmune diseases, and where antibodies or TCRs recognizing pathogenic antigens could also cross-react against self-antigens (42). Evidences supporting the validity of neoantigenic molecular mimicry in the onset of irAEs come from observations in the cancer context that (1) TCRs reactive to certain neoantigens exhibited cross-reactivity to the wild-type peptides (43), and (2) shared T-cell clones were identified between tumors and target tissues of irAEs from ICI-treated patients in whom irAEs developed (44, 45).

Nonetheless, as suggested in our prediction model, it was not sufficient for immunogenic neoantigens alone to trigger irAEs, but abundant DC were required. As the most potent antigen-presenting cell type, DC are critical for priming naive T-cells by presenting antigens *via* major histocompatibility complex molecules and providing costimulatory signal (46). The engagement of DC in triggering autoimmunity has been documented *via* various mechanisms (47). For instance, deficient apoptosis of DC may increase DC numbers and lead to the onset of systemic autoimmunity (48). Additionally, previous studies suggest that DC may transfer the majority of tumor antigens from tumors to draining lymph nodes for the purpose of efficient priming of T-cells (49–51). Thus, a possible mechanism whereby neoantigenic mimicry may be implemented is that, intratumoral DC locally capture immunogenic neoantigens in tumor microenvironment, and subsequently migrate to draining lymph nodes where they disseminate neoantigens and stimulate resident T-cells. Upon being educated by DC, these T-cells would circulate systemically to induce neoantigen-specific immunopathologies such as irAEs against the cross-reactive self-antigen at distal sites.

Given the similarity between the irAEs and that of a chronic graft-versus-host-disease (GVHD) reaction following allogenic hematopoietic stem cell transplantation, there is a new theory for ICIs-induced irAEs (52). It was hypothesized that ICIs induced a graft-versus-malignancy effect, which eradicated metastatic cancer in a minority of patients, but also involved an auto-GVHD reaction that leaded to widespread autoimmunity in the majority. Based on this theory, an off-label low-dose nivolumab plus ipilimumab regimen was developed and tested in 131 unselected stage IV cancer patients (53). The irAEs profile of this combined low-dose treatment was significantly safer than that of the established protocols without compromising efficacy. Our finding that DC abundance showed the strongest correlation with irAEs risk supports the auto-GVHD reaction theory as host-derived DC are also important to elicit allogeneic T cell responses (54).

Our model also highlights the potential role of $CD4^+$ naive T-cells in tumor microenvironment in promoting irAEs developement. The recruitment of $CD4^+$ naive T-cells into non-lymphoid tissues, including tumors, has been reported (55, 56). although their biological significances remain uncertain. It is notable that $CD4^+$ T-cells are of fundamental importance in mediating autoimmunity. And this role is achieved *via* the differentiation of $CD4^+$ naive T-cells into various lineages of T helper cells, depending on external

cytokine microenvironment and transcription factors they induce (57).

Further performance enhancement (unexplained variance = 0.24) was seen in the composite model comprising 3 novel molecular predictors (*IRF4*, *TCL1A*, and SHC-pY317). All these features have been implicated in immune regulation (28–33, 38). Importantly, we observed elevated expression level of IRF4 in ICI-treated melanoma patients with irAEs. IRF4 is a member of the interferon regulatory factor family of transcription factors and selectively expressed in lymphoid and myeloid cells. IRF4 deletion in mice may induce transplant acceptance by establishing $CD4^+$ T-cells dysfunction (58) and render mice resistant to several autoimmune diseases (28), such as ulcerative colitis and experimental autoimmune encephalomyelitis. Intriguingly, a MEK1/2 inhibitor trametinib was capable of inhibiting IRF4 expression in activated $CD4^+$ T-cells (58). Collectively, these evidences suggest the therapeutic potential of targeting IRF4 expression for abrogating inflammatory toxicities from immune checkpoint blockade.

MicroRNAs are critical posttranscriptional regulators of target genes expression, and the number of microRNAs implicated in immune disorders like autoimmunity has increased dramatically (40). A recent study has shown that microRNA-146a may regulate irAEs by ICIs in mice (8). Of note, we found 6 microRNAs predictive of irAEs risk. Given that miRNAs act by targeting multiple genes within a pathway, thus causing a broader yet specific response (59), our finding may further spark the possibility of using microRNAs as therapeutics for irAEs with multifactorial origin.

We also noted a study profiled for serum cytokines/chemokines in 47 cancer patients with ICIs treatment (60). It revealed that patients with irAEs had lower baseline levels and higher posttreatment elevation in serum IFN-gamma-inducible small cytokines (CXCL9 and CXCL10). In our work, the IFN-gamma signature in tumor microenvironment showed positive correlation with irAEs risk. This observation may be associated with the difference between circulating and tumor immune microenvironment, and deserve further investigation.

This study has several limitations. First, FAERS is a spontaneous reporting database which may include reporting bias and inaccurate reports, although it has previously been used to determine risk factors linked to the development of irAEs (9, 61). Second, cancer patients with more responsive tumor immune microenvironment may remain on ICI treatment longer. However, the majority of irAEs reported during anti–PD-1 therapy occur within the first few months of commencing treatment (62), which implys that treatment duration may not bias our results. Given the identification of markers (e.g., TMB, immune signatures, and PD-L1 expression) predictive of both ICI response and irAEs risk in our study, we propose that the association between response and irAEs risk may be partially linked *via* high tumor immunogenicity and immunoresponsive microenvironment represented by these predictors. Therefore, it is necessary to discern markers able to discriminate anti–tumor efficacy from the risk of irAEs in patients with ICI treatment. Notably, all 11 novel molecular features in our study have not been reported to be associated with anti–tumor efficacy. Third, in addition to cancer-associated immunogenomic features reported in our study, host features, such as age, genetic susceptibility to autoimmunity, pre-existing autoimmune disease, and gut microbiome, may influence the development of irAEs (6). Fourth, further experimental study is required to classify the biological significance of novel features identified in our study.

In conclusion, our approach allowed us to identify cellular and molecular candidates as well as their optimal combinations for identifying patients with the risk of irAEs development during anti–PD-1 therapy, irrespective of cancer types. These findings may advance our understanding of mechanisms that drive irAEs development and tailoring personalized surveillance strategies.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

YS, XH, and LZ conceived the concept and designed the study. LZ conducted statistical analysis. LZ drafted the manuscript. YS and XH performed the critical revision of the manuscript for important intellectual content. YS and XH obtained funding and supervised the study. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.1032221/full#supplementary-material

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Immune-related adverse events: A bibliometric analysis

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Background: Despite providing clinical benefit, immune checkpoint inhibitors (ICIs) can cause immune-related adverse events (irAEs) in a number of patients. This study explored the development pattern in irAEs research from a bibliometric perspective.

Methods: We obtained articles and reviews related to irAEs from the Web of Science Core Collection (WoSCC) (retrieved on September 13, 2022). Using the R package "Bibliometrix", the main bibliometric features were calculated, and a three-filed plot was generated to show the relationship between authors, institutions, and topics. VOSviewer was used for co-authorship and keyword co-occurrence analysis and visualization. CiteSpace was used to detect burst references and keywords.

Results: A total of 3995 publications on irAEs were included. The United States (US), Japan, and China had the highest publications. The Journal for ImmunoTherapy of Cancer had the highest number of publications. In addition to "immune-related adverse events", "immune checkpoint inhibitors", "immunotherapy", and "nivolumab" were the most frequently used keywords.

Conclusions: A bibliometric analysis of 17 years of irAEs research was conducted to map a basic knowledge structure including countries, institutions, authors, journals, and publications. The findings provided a comprehensive perspective on the broad future of this research area.

KEYWORDS

immune-related adverse events, bibliometrics, VOSviewer, citespace, frontiers

1 Introduction

Immune checkpoint inhibitors (ICIs) have emerged as one of the novel and practical approaches to cancer therapy, providing tremendous clinical benefits to patients with cancer. ICIs can damage self-tissue while killing tumors, resulting in a series of toxic side effects that we call immune-related adverse events (irAEs) (1). Due to the prevalence of

ICIs, about 54%-76% of cancer patients experience irAEs, including severe toxic reactions (e.g., myocarditis) or permanent toxic reactions (e.g., autoimmune diabetes) (2). Therefore, there is an increasing emphasis on the research, diagnosis, and management of irAEs. However, conducting clinical studies on a large scale is difficult due to the significant heterogeneity of irAEs (3). Even though there are more and more publications on irAEs, a complete analysis of publications, countries, institutions, journals, authors, and keywords is still lacking.

Pritchard introduced bibliometrics in 1969, which was defined as "the application of mathematical and statistical methods to the computation and analysis of different aspects of textual information to reveal the processes of textual information and the nature and trends in the development of a discipline (4)." Currently, bibliometrics is widely used to investigate the characteristics of academic publications (5). For example, it identifies the most influential countries, journals, institutions, and authors in a research field (6). It helps researchers identify high-frequency cited publications and keywords. It also helps visualize and analyze the collaboration between countries, institutions, and authors (7). In addition, bibliometrics can help researchers quickly grasp a specific research field's evolution and research frontiers. Several bibliometric analyses have investigated the trends and hot topics in the field of immunotherapy (8–10). In the bibliometric analyses targeting immunotherapy in hepatocellular carcinoma and colorectal cancer, both irAEs were found to be an essential topics.

Furthermore, a bibliometric study of 11,971 publications on ICIs from 2000 to 2020 revealed that irAEs formed a unique cluster in the keyword co-occurrence analysis (11). This indicates that irAEs are becoming a widely followed issue in immunotherapy. The bibliometric analysis of irAEs, however, has not been published yet. The purpose of this bibliometric analysis was to fill this gap by creating a global knowledge mapping of scientific publications about irAEs.

2 Methods

2.1 Data source and publication search strategy

Web of Science (WoS) incorporates over 12,000 journals and is one of the most frequently accessed academic databases (12). When the bibliometric analysis was performed against other databases such as Scopus, Medline, and PubMed, WoS emerged as the most comprehensive and reliable (13). In the present research, the relevant publications were searched and exported to the Web of Science Core Collection database (WoSCC) on September 13, 2022. All versions of WoSCC were used for the study. After consultation with our senior literature search experts and agreement by all authors, the search strategy was set as follows: [TS = (Immune-related side effect OR Immune-related side effects OR Immune-related adverse reaction OR Immune-related adverse reactions OR Immune-related adverse reactions OR Immune-related adverse effect OR Immune-related adverse effects OR Immune-related adverse event OR Immune-related adverse events OR Immune-related toxicity OR Immune-related toxicities)]. The type of publication included regular and review articles. The publication language was restricted to English to facilitate further literature content analysis. For further analysis, relevant publications were extracted and saved in plain.txt format (including complete records and cited references) (14).

2.2 Software tools for performing bibliometric analysis in this study

This study used R version 4.0.1 (15), VOSviewer (16), and CiteSpace (17) for bibliometric analysis.

In bibliometrics and scientometrics, the Bibliometrix R package provides tools for quantitative research. In Bibliometrix, authors are extracted from the AU field, including all authors. Keywords are extracted from the DE field, citations from the TC field, and the year of publication from the PY field.

In this study, Bibliometrix version 4.0.0 was used to

1. Count basic bibliometric metrics such as the number of publications and citations,
2. Determine the frequency of keywords/terms,
3. Calculate the frequency of collaboration between countries, and
4. Visualize a three-field plot for keywords plus analysis.

Bibliometric networks can be constructed and visualized using the VOSviewer software tool (18). Based on the software's embedded clustering algorithm, VOSviewer can construct and visualize co-occurrence networks of important terms extracted from the scientific literature (19). In addition, VOSviewer supports overlaying visual maps to show the network over time. In this study, we primarily utilized co-authorship analysis and co-occurrence analysis. On the one hand, co-authorship networks were constructed to explore the collaborative relationships between authors and their institutions (20). Alternatively, the co-occurrence network shows how the authors' keywords are related (21).

CiteSpace is a citation visualization and analysis software. Since the structure, patterns, and distribution of scientific knowledge are presented through visualization, the visualization obtained through this method is also called "scientific knowledge mapping (17)." In this study, it was used

to identify highly cited references and keywords that experienced high citation bursts during a particular period.

Additionally, an international collaboration between countries was visualized using the online bibliometric website (https://bibliometric.com/). An exponential growth function in Excel was used to analyze the number of publications published per year.

3 Results

3.1 General analysis of publication status

An overview of the study can be found in Figure 1. There were 3995 publications on irAEs, including 2,744 regular and 1251 review papers. Figure 2 demonstrates the annual number of publications related to irAEs and the cumulative number. A 46.44% annual growth rate was observed. Supplementary Figure 1 shows the percentage of publication types across years, countries, and authors. Research articles dominate in all dimensions. In 2005, the first article was published in Leukemia. Plumas et al. firsty revealed that mesenchymal stem cells (MSCs) could induce apoptosis in activated T cells, which they indicated could help to develop approaches to control irAEs (22). Overall, the cumulative number of publications steadily increased from 1 in 2005 to 133 in 2014. In the following seven years, the number of publications proliferated until 2021, when the cumulative number of publications reached 3340. In addition, the relationship between the number of publications per year and the year of publication was assessed using an exponential growth model, which matched the trend in the number of publications per year (R^2 = 0.9819).

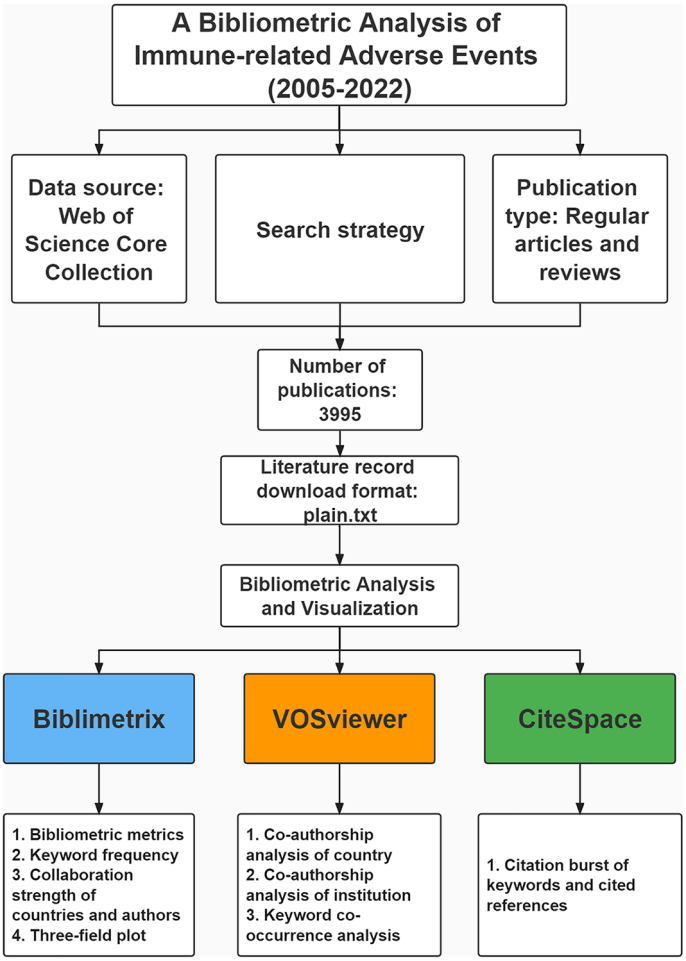


FIGURE 1
Process and key steps of the study.

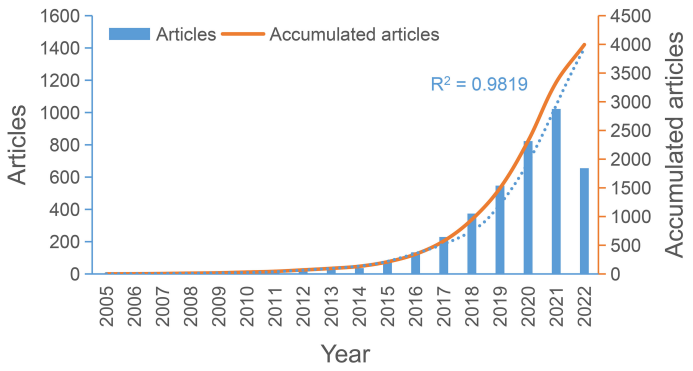


FIGURE 2
The number of publications per year and the cumulative number.

3.2 Analysis of national publications volume

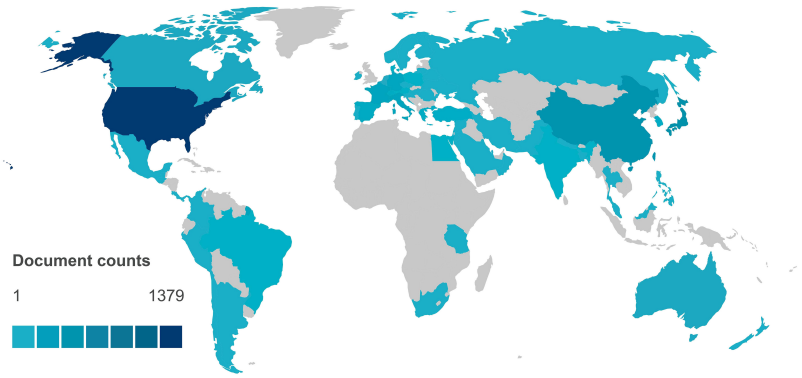
In order to explore the countries/regions that contributed the most in the field, an analysis of the number of national publications was conducted. The results are presented in Figure 3. The United States (US) ranked first with 1379 publications. It was followed by Japan (592), China (505), and Italy (218). The remaining countries/regions had less than 200 publications.

To further investigate the collaborative relationships between countries/regions, we visualized the country/region collaborations in Supplementary Figure 2. The results indicated that the research in the field of irAEs was dominated by the US. The most frequent collaboration was between the US and France (frequency = 75). The following countries were Italy (frequency = 73), the United Kingdom (UK) (frequency = 68), and China (frequency = 67). All of these national collaborators were from the US.

3.3 Analysis of institutional publications volume

To explore the contribution of institutions to the field of irAEs, we analyzed the number of institutional publications. Globally, approximately 4,060 institutions conducted irAEs-related research. The top 20 research institutions are summarized in Figure 4. There were 15 US institutions, 3 Chinese institutions, 1 French institution, and 1 Australian institution. The University of Texas MD Anderson Cancer Center ranked first with 457 publications.

A co-authorship analysis was performed on all publications to investigate inter-institutional collaborations further. In the



Country	Articles
USA	1379
JAPAN	592
CHINA	505
ITALY	218
FRANCE	195
GERMANY	124
AUSTRALIA	118
UNITED KINGDOM	115
CANADA	95
SPAIN	89

FIGURE 3
An overview of the contributions of each country based on the number of publications.

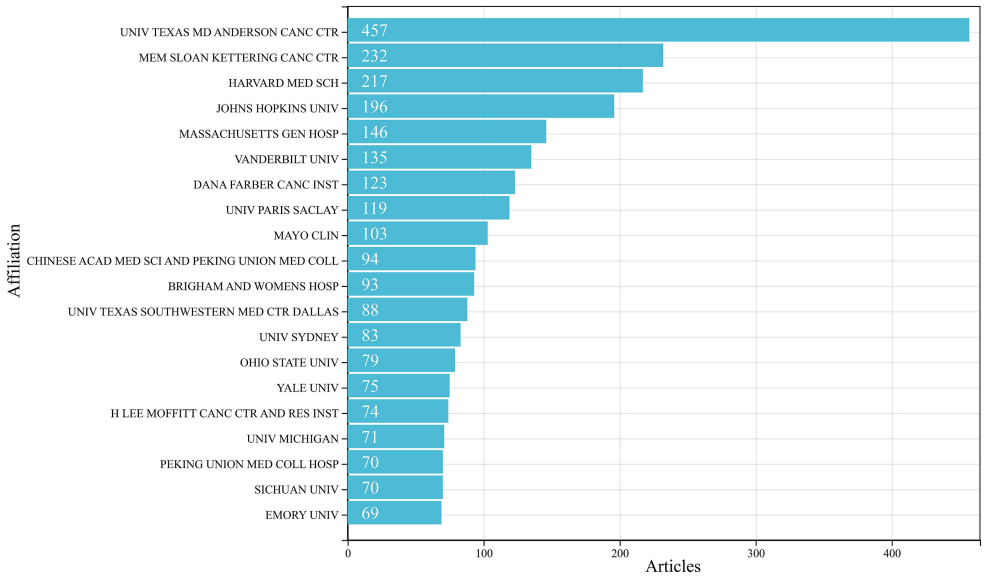


FIGURE 4
The top 20 institutions with the most publications in the field of irAEs.

clustering network for the co-authorship analysis and the time-overlapping network, the size of the circles indicate the number of publications. In the clustering network, the color of the circles represented the research groups automatically classified according to the intensity of collaboration. In the time-overlapping network, the circle's color represented the average year of publication start for each institution in the particular research area. As shown in Supplementary Figure 3A, 119 institutions were identified as having published at least 15 articles. One hundred nineteen institutions formed a total of 8 clusters. The red color refers to the cluster containing the most institutions, with 30 institutions belonging to this cluster, most of which were US institutions. In Supplementary Figure 3B, research institutions represented by MD Anderson Cancer Institute were early starters in the field of irAEs. In contrast, researchers in China and Japan conducted relatively new research in this area.

3.4 An analysis of the number of publications and impact of journals

The 3995 publications included in the research were published in 943 journals. The top 10 journals and their latest impact factors (IF) were listed in Table 1, sorted by the number of publications. Five of the top 10 journals were classified in Journal Citation Reports (JCR) Quartile 1 (Q1). Three publishers each from the US, UK, and Switzerland, and one other publisher from Egypt.

3.5 Author influence analysis

A total of 20,734 authors participated in irAEs-related studies. As demonstrated in Table 2, Johnson DB was the most productive author with 47 articles and H-index of 22. He was followed by Wolchok JD (40 publications, H-index=30) and Zhang L (37 publications, H-index=9).

Supplementary Figure 4A illustrates the clustering diagram of collaborative relationships among researchers. The circle size represents the number of publications, and the color represents the clusters. Seventy-seven authors with several publications greater than or equal to 10 were clustered into 10 clusters. Three clusters were scattered outside of a larger community consisting of 7 clusters. There were no collaborative relationships between the different communities. It suggested that collaboration between research teams/labs conducting research related to irAEs must be further strengthened. Supplementary Figure 4B depicts the time-overlapping network of clustering results. We observed that researchers from China, represented by Mengzhao Wang, were forming new research networks on irAEs. Given that collaboration

among different research groups are insufficient, national and inter-institutional collaboration is one of the future directions.

3.6 Research hotspot analysis

3.6.1 Most cited publications

The frequency of citations in a particular field can indicate research impact; citation counts can be used to assess the most cited articles. Supplementary Table 1 lists the ten most cited publications between 2010 and 2018, 60% of which have been cited more than 1000 times.

The most cited article was published in 2010 and was titled "Improved Survival with Ipilimumab in Patients with Metastatic Melanoma (23)." The study reported the survival of patients with metastatic melanoma treated with ipilimumab plus gp100 and the probability and severity of irAEs. The authors further pointed out that appropriate treatment could improve most irAEs. The second most cited publication was also published in The New England Journal of Medicine. Postow et al. published a review entitled "Immune-Related Adverse Events Associated with Immune Checkpoint Blockade" in 2018. In this review, the authors focused on ten critical questions about immunotherapy, for example, whether the occurrence of irAEs is related to the effectiveness of treatment with ICIs, providing a valuable reference for researchers to understand irAEs (24).

3.6.2 Reference citation burst analysis

Supplementary Figure 5 illustrates the burst of the top 20 most cited references. The minimum duration of the burst is two years. The blue line represented the observed time interval from

TABLE 1 Top 10 journals with most publications in the field of immune-related adverse events.

Rank	Sources	Articles	Country	IF	JCR-c
1	JOURNAL FOR IMMUNOTHERAPY OF CANCER	182	UK	12.469	Q1
2	FRONTIERS IN ONCOLOGY	116	Switzerland	5.738	Q2
3	FRONTIERS IN IMMUNOLOGY	94	Switzerland	8.786	Q1
4	CANCERS	80	Switzerland	6.575	Q1
5	JOURNAL OF IMMUNOTHERAPY	76	US	4.912	Q2
6	EUROPEAN JOURNAL OF CANCER	67	Egypt	10.002	Q1
7	CANCER IMMUNOLOGY IMMUNOTHERAPY	61	US	6.63	Q1
8	ONCOLOGIST	57	US	5.837	Q2
9	IMMUNOTHERAPY	55	UK	4.04	Q3
10	JOURNAL OF ONCOLOGY PHARMACY PRACTICE	52	UK	1.416	Q4

TABLE 2 Top 10 authors with the most publications in the field of immune-related adverse events.

Rank	Authors	Articles	H-index
1	JOHNSON DB	47	22
2	WOLCHOK JD	40	30
3	ZHANG L	37	9
4	NAIDOO J	35	21
5	ROBERT C	34	26
6	HODI FS	34	24
7	LAMBOTTE O	32	20
8	REYNOLDS KL	31	14
9	MARABELLE A	29	19
10	MICHOT JM	29	19

2005 to 2022, while the red line represented the duration of the burst. The article "Improved Survival with Ipilimumab in Patients with Metastatic Melanoma," published in The New England Journal of Medicine, had the strongest citation burst value (citation burst = 148.18) between 2011 to 2018 (23). In addition, citation bursts continued for four articles, including "Management of Immune-Related Adverse Events in Patients Treated With Immune Checkpoint Inhibitor Therapy: American Society of Clinical Oncology Clinical Practice Guideline," which had the highest burst value of 67.11 (25). This article is a practice guideline of the American Society of Clinical Oncology Clinical and has contributed to the management of irAEs. The second most popular article was "Fatal toxic effects associated with immune checkpoint inhibitors: a systematic review and meta-analysis." Wang et al. reported the incidence and timing of fatal toxic effects associated with ICIs (26). In the future, this type of research topic may remain popular and become a potential frontier in the research on irAEs.

3.6.3 Frequency of keyword occurrence and clustering analysis

The minimum number of occurrences was set to 20, and 68 of the 4865 keywords met the criteria and were included in the analysis. The keywords were combined if they had similar meanings. The network visualization of these keywords is shown in Figure 5A. Node size reflects keyword frequency, while the distance between nodes indicates the strength of their relationship. The 68 keywords were divided into six clusters, reflecting the critical topics in the field of irAEs research. Keywords that are more closely related were assigned to the same cluster. Cluster 1 was red, and the primary keywords focused on the manifestation of irAEs in different systems, such as "acute kidney injury," "arthritis," "myocarditis," and "encephalitis." Cluster 2 was green and focused on various widely used ICIs with the main keywords "anti-ctla-4", "anti-pd-1", "anti-pd-l1" and "efficacy." In addition, some terms, such

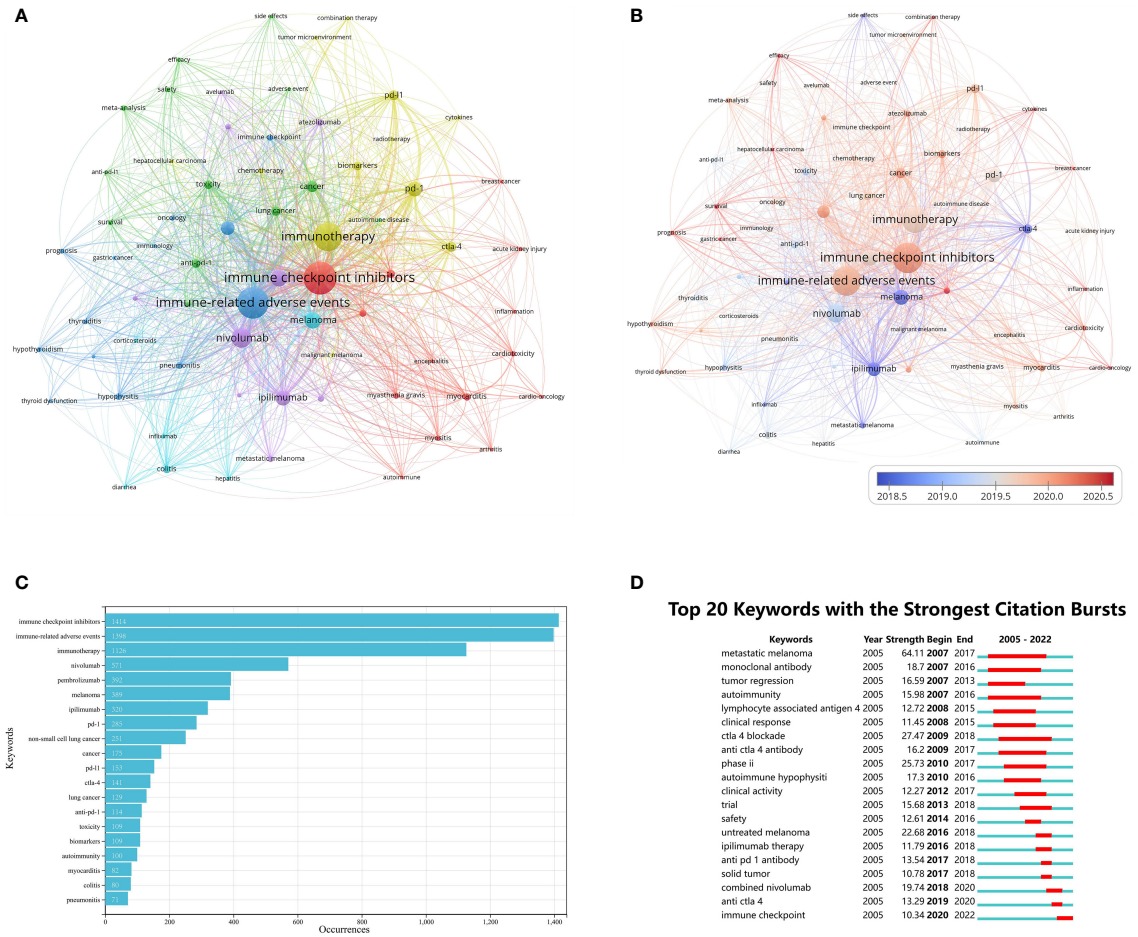


FIGURE 5
Research hotspots on irAEs **(A)** keyword co-occurrence network of authors; **(B)** time-overlapping co-occurrence analysis network of author keywords; **(C)** a list of the 20 most frequently used keywords; **(D)** the 20 keywords with the strongest citation bursts).

as "safety" and "survival," were included in Cluster 2. Cluster 3 was in blue and concentrated on the description of irAEs with various cancers, with the main keywords being "immune-related adverse events," "non-small cell lung cancer," and "oncology." Cluster 4 in yellow focused on different immune checkpoints and potential biomarkers, mainly involving "pd-1", "pd-l1", "ctla-4", "cytokines," and "biomarkers." Cluster 5 in purple mainly comprised a variety of ICIs that have been approved by the Food and Drug Administration (FDA), such as "atezolizumab," "durvalumab," "ipilimumab," and "nivolumab." The sixth cluster in light blue included primarily "colitis," "diarrhea," "hepatitis," and "infliximab." It seems to be about irAEs of the digestive system and treatment. Figure 5B illustrates the time-overlapping visualization of the authors' keywords. Earlier appearing keywords were presented in blue, while red indicated recent keywords. Early periods of research focused primarily on "melanoma," "metastatic melanoma," "ipilimumab," and "ctla-4." In contrast, recent research has focused on the topics of "combination therapy," "efficacy," "hepatocellular carcinoma," "gastric cancer," "tumor microenvironment," and "cytokines."

Figure 5C shows the top 20 keywords in order of frequency of occurrence, where " immune checkpoint inhibitors" was the most frequently used keyword with 1414 occurrences, followed by "immune-related adverse events" (N = 1398) and "immunotherapy" (N = 1126). Among the top 20 keywords, "non-small cell lung cancer" (N = 251) and "lung cancer" (N = 129) were the only cancer types that appeared. Figure 6 further demonstrates the association between authors, institutions, and keywords in the field of irAEs research.

3.6.4 Keywords citation burst analysis

In Figure 5D, we present the top 20 keywords with the most robust citation bursts, with a minimum duration of one year. The keywords "metastatic melanoma" (2007–2017), "monoclonal antibody" (2007–2016), and "autoimmunity" have received the most protracted attention over time. While keywords such as "combined nivolumab" (2019–2020), "anti ctla 4" (2019–2020), and "immune checkpoint" (2020–2022) have been used more recently, indicating that these keywords have attracted enough attention to become popular research topics in the future.

4 Discussion

The present research analyzed the growth pattern of irAEs-related studies from 2005 to 2022 using a bibliometric approach. The growth trend of irAEs-related research could be divided into 2 phases according to whether the annual publications exceeded fifty. Before 2015 was a slow growth phase with less than 50 publications per year. From 2015 onwards, irAEs-related studies entered a rapid growth phase, with the annual number of publications exceeding more than fifty each year. Until 2021, the annual publication volume reached 1022 publications. It indicates that irAEs-related research has started to enter a rapid development stage. The potential reason might be that with the widespread ICIs in oncology treatment, the incidence of irAEs increased, and people began to realize that poor irAEs control might affect patients' benefits (27). As a result, research institutions have been increasing their support for research

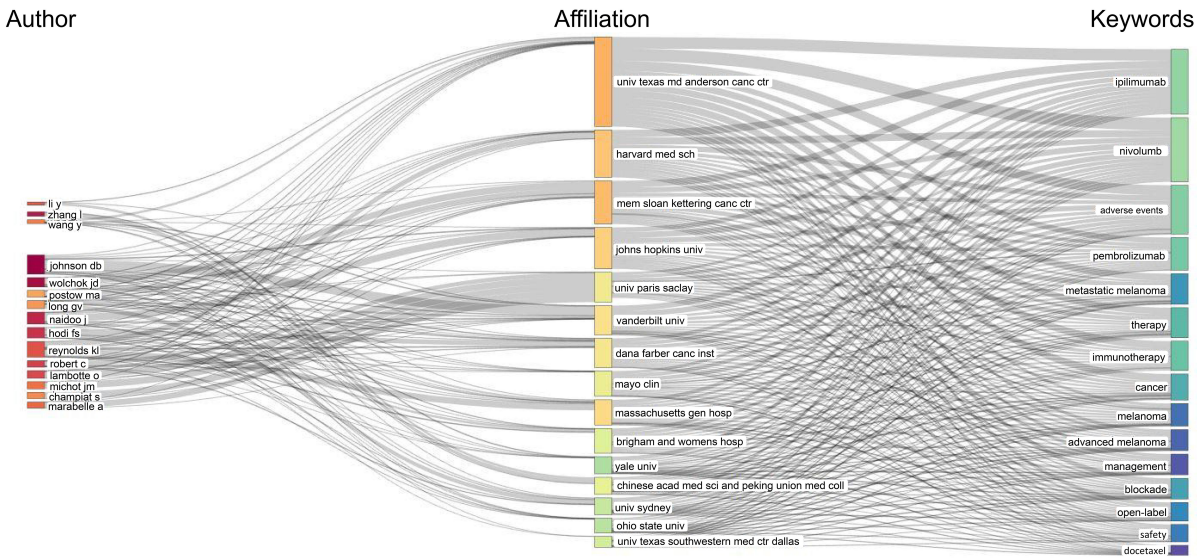


FIGURE 6
Three-field plot of the keywords plus analysis on irAEs (left field: authors; middle field: affiliations; right field: keywords plus).

related to irAEs, and research funding has been increasing, contributing to the high growth rate of the field.

For this research, the top 10 countries published 3430 articles, accounting for 85.9% of the total publications. Developed countries, represented by the US and Japan, dominate these ten countries. China is the only developing country. In addition, the US also dominated international collaborations, with US-centered international collaborations occupying eight positions among the top 10 countries in terms of frequency of collaboration. The above findings further confirmed the vital contribution and leadership of the US in the field of irAEs research. It could be related to the favorable national economic situation of the US with high investment in health care. Extensive international collaborations will be beneficial to the development of the field and the improvement of the overall research level.

Similar to the national distribution of the number of publications, fifteen of the top twenty institutions were in the US. Although ranked 3rd in terms of the number of publications, China had only three institutions in the top 20 of the list. Japan, which ranked 2nd in terms of the number of publications, had no institution in the top 20. In contrast, a French institution ranked 7th with 119 publications. Most of these studies were based on international collaborations, suggesting that seeking extensive collaboration among institutions might be essential to improve research competitiveness when economic or resources are limited.

Peer-reviewed journals are an essential carrier of scholarly publications. Core journals often bear the task of publishing necessary research in the field (28). By analyzing the number of journal publications, we could identify the top journals in the field of irAEs and provide researchers with potential journals to submit. In the field of irAEs research, the top 10 journals have several publications greater than 50. Among them, The Journal for ImmunoTherapy of Cancer has the highest number of publications, with 182 publications. The most significant publications were Frontiers in Oncology (116) and Frontiers in Immunology (94). The impact factor and JCR were essential indicators to evaluate the impact of journals (29). JCR quadratically divided all journals into zones 1,2,3,4 based on the impact factor (30). Among the top 10 journals in terms of the number of publications, Q1 journals account for 50%. Furthermore, although Japan and China contributed significantly to irAEs research, there was a lack of Asian publishers among the top 10 ranked journals. It suggests a need to establish and develop journals with international influence in Asia.

Research hotspots represented scientific topics widely followed by researchers in a specific period and were one of the questions this study tried to answer. The number of citations could be one of the indicators of how influential a scholarly publication was (31). Highly cited publications tend to represent essential topics in the study field. By calculating the number of citations, we could identify the highly cited publications and thus identify research hotspots. The ten most cited publications identified in this study were published from 2010 to 2018 and focused on the clinical manifestations of irAEs and how to manage irAEs effectively. Of the top 10 cited publications, the earliest two were published in 2010.

Interestingly, both studies were about the application of ipilimumab in the treatment of melanoma. Stephen Hodi et al. demonstrated in a phase 3 clinical trial that ipilimumab improved overall survival in patients with previously treated metastatic melanoma (23). In this study, the incidence of grade 3 or 4 irAEs was 10-15%. Wolchok D et al. explored the optimal dose of ipilimumab alone for advanced melanoma in another phase 2 clinical trial. In this study, the authors reported that the incidence and severity of irAEs increased with increasing doses of ipilimumab. The most common grade 3-4 irAEs were gastrointestinal (32). These two critical studies initiated the application of ipilimumab to treat metastatic melanoma.

Subsequently, Weber et al. published a review in 2012 that systematically described the symptoms of irAEs caused by ipilimumab and management strategies (33). It provided an essential reference for oncologists. In 2014, after the first CTLA-4 monoclonal antibody was approved by the US FDA (34), the PD-1 monoclonal antibody was again approved by the FDA for the treatment of metastatic melanoma (35). Between 2015 and 2017, three reviews on immune checkpoint blockade were published, extensively describing the clinical presentation and management of irAEs associated with immune checkpoint blockade (CTLA-4, PD-1, PD-L1) (36–38). The two cases of fulminant myocarditis reported by Johnson et al. also drew widespread attention from researchers on lethal irAEs (39). In addition, Gibney et al. published a review entitled “Predictive biomarkers for checkpoint inhibitor-based immunotherapy” in 2016, highlighting the value of predictive biomarkers in improving the efficacy of immune checkpoint inhibitor therapy (40). Researchers conducted extensive studies on this topic and identified promising biomarkers in various cancer types.

With the widespread use of ICIs, understanding irAEs has gradually improved. Recently, the American Society of Clinical Oncology published a clinical practice guideline for irAEs, which further standardized the management of irAEs (25). Based on sufficient experience in clinical practice, Postow et al. pointed out that the key to improving the treatment of irAEs lies in elucidating the mechanisms of occurrence to develop more effective treatments. Furthermore, the authors raised a critical issue in this review, namely whether the occurrence of irAEs was correlated with the efficacy of ICIs (24). Patients with irAEs have

been found to have higher response rates and better outcomes than patients without such events (41). Although these findings require universal verification, the rational management of irAEs for optimal efficacy is a goal that investigators should strive to achieve. We are pleased to note the results of the research by Kleef et al. They attempted to treat advanced cancer patients with a combination of low-dose ICIs (42). According to the study's results, low-dose ipilimumab (0.3 mg/kg) plus nivolumab (0.5 mg/kg) had a better irAE profile than the regular regimen without compromising efficacy. As Bakacs et al. suggest, modest activation of the immune system by low doses of ICIs may achieve comparable efficacy with less irAE risk (43). As keywords reflected the core content of the research, co-occurrence analysis could identify high-frequency keywords that appeared simultaneously in different studies. These keywords usually represented the focus of the research field. This study's most frequently used keywords were "immune checkpoint inhibitors" and "immune-related adverse events." In addition, other keywords focused on the use of "immune checkpoint inhibitors" in different cancer types and organ-specific irAEs. In addition, "biomarkers" was another frequently occurring keyword. Several studies reported the role of biomarkers in predicting the efficacy of ICIs treatment, disease progression, and recurrence patterns (44–46). However, there were few studies on biomarkers related to irAEs. Since severe irAEs might interrupt treatment, combining approaches to explore biomarkers related to irAEs could provide a powerful tool to maximize individual treatment efficacy.

Burst detection is a bibliometric analysis method provided by CiteSpace. Its primary function is to identify keywords or cited references that appear to have significant shifts over a specific period. Keywords and cited references with burst characteristics imply that they have been widely followed and discussed. It can provide a reference for researchers to explore research hotspots. In this study, "immune checkpoint" was a keyword with a continuous burst since 2020. In addition, there were four cited references in 2020, and the burst has continued. Three of these reviews focused on the management of irAEs (24–26). One study reported five-year survival and the frequency and intensity of adverse events for the combination of nivolumab and ipilimumab in advanced melanoma (47). This is consistent with the development of immune checkpoints and their inhibitors. In light of the above burst detection results, the pathogenetic characteristics of ICIs-associated irAEs and management strategies may be a research direction of interest in the coming period.

We acknowledge that this study has some modest limitations. First, only articles and reviews written in English and recorded in the WoSCC database were included in this study. While this approach may have overlooked some valuable studies, given that WoSCC is the most commonly used database for scientometric analysis and covers the vast majority of studies, we do not believe this will substantially impact overall trends. Second, due to the delay in citation volume, recently published high-quality studies may not have received the attention they deserve and will need to be updated accordingly in subsequent studies. Nevertheless, this study will significantly help relevant researchers to understand the developments, hot spots, trends, and frontiers of irAEs and to identify areas where further research is still needed.

5 Conclusion

In conclusion, research on irAEs related to ICIs has received growing attention. The significant increase in annual publications indicates this research area's growing importance, with the most significant number of publications in the US. The study identified the top researchers and institutions involved in irAE research worldwide. The Journal for ImmunoTherapy of Cancer is the most active in this research field. Wolchok is the most influential author. The pathogenetic characteristics of irAEs and strategies for management were considered hot topics, and the molecular mechanisms by which irAEs occur may be a key direction for future research. As a result, new researchers and policymakers are provided with a comprehensive overview of the field's evolution and frontiers.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

XL, Y-YX, and X-TS conceived and designed the study. S-TJ wrote the manuscript and participated in the design of the study. S-TJ and Y-GL were responsible for analysis and network construction. LZ performed the data analysis and data interpretation. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Immune-related ureteritis and cystitis induced by immune checkpoint inhibitors: Case report and literature review

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Immune checkpoint inhibitors (ICIs), including anti-cytotoxic T lymphocyte-associated protein 4 (anti-CTLA4) and anti-programmed death cell protein 1 (anti-PD-1), are increasingly prescribed in metastatic carcinoma therapy. ICI-related kidney injury is gradually recognized by clinicians. However, immune-related ureteritis and cystitis easily go undiagnosed. We report three cases of PD-1 monoclonal antibody (mAb)-related ureteritis and cystitis. We further carried out a review of the literature about ICI-related ureteritis and cystitis. The cases in our reports manifest urinary irritation, sterile pyuria, gross hematuria, hydronephrosis, dilation of the ureters, and acute kidney injury. Urinary irritation improved effectively; urinalysis and renal function returned to normal after glucocorticoid therapy. During ICI therapy, urinalysis and renal function and urinary imaging examination are recommended to be monitored regularly. It contributes to identify immune-related ureteritis/cystitis earlier to efficiently alleviate urinary symptoms and immunologic urinary tract injury through glucocorticoid therapy while avoiding the abuse of antibiotics.

KEYWORDS

immune checkpoint inhibitors, immune-related adverse, glucocorticiods, case report, literature review

Introduction

Immune checkpoint inhibitors (ICIs), which disinhibit T-cell cytotoxicity against cancer *via* blocking cytotoxic T lymphocyte-associated protein 4 (CTLA-4), programmed death cell protein 1 (PD-1), or programmed death ligand-1 (PD-L1), are known to activate immunoactivity against malignancies (1, 2). ICIs include anti-CTLA-4, anti-PD-L1, and anti-PD-1 antibodies. As ICIs become more prevalent in cancer therapy, new and rare immune-related adverse events (irAEs) gradually attract the attention of medical

oncologists. ICI-related acute kidney injury has been noticed in recent years, including acute interstitial nephritis, glomerulopathy (minimal change disease, focal segmental glomerulosclerosis, C3 nephropathy), and immune-related diseases (lupus nephritis, vasculitis, and thrombotic microangiopathy) (3–6).

However, irAEs involving the bladder and urinary tract are rarely reported and ignored by medical oncologists. The differential diagnosis of immune-related cystitis mainly includes bacterial cystitis, metastasis, radiation cystitis, and cystitis caused by other drugs. Obstructive nephropathy caused by renal calculus, carcinoma infiltration, and tuberculosis are excluded through CT scan. The diagnosis poses a challenge correspondingly. The patients usually received multiple courses of unnecessary antibiotics before getting an accurate diagnosis and treatment.

We reported three cases of immune-related ureteritis and cystitis, which were induced by ICIs [PD-1 monoclonal antibody (mAb)]. We further performed a review of the literature about ICI-related ureteritis and cystitis (Table 1). It contributes to identify immune-related ureteritis/cystitis earlier to efficiently alleviate urinary symptoms and immunologic urinary tract injury through glucocorticoid therapy while avoiding the abuse of antibiotics.

Case 1

A 49-year-old man was diagnosed as having esophageal carcinoma (PT3N3M0, Stage IV) in December 2020. Radical resection of midpiece esophageal carcinoma was performed, and chemotherapy with "paclitaxel (albumin-bound) + oxaliplatin" was given for four courses. In November 2021, chemotherapy combination with immunotherapy (tislelizumab 200 mg on day 1 + docetaxel 100 mg on day 2 + nedaplatin 100 mg on day 3) was given due to multiple lymph node metastasis in the mediastinum and posterior peritoneum. The date of the last chemotherapy was 28 June 2022. He did not have a medical history of hypertension, diabetes, or kidney disease.

He complained of gross hematuria, pollakiuria, painful micturition, and low back pain after six courses of tislelizumab (PD-1 mAb). Physical examination: T (Temperature) 36.4°C, P (Pulse) 85 bpm(beats per minute), R (Respiration) 16 bpm (breaths per minute), BP (Blood pressure) 125/75 mmHg. The abdomen was soft, no tenderness and rebound. His bilateral renal percussive pain was positive. There was no edema in the lower limbs.

Urinalysis showed red blood cells (RBCs) of 4,932/μl, white blood cells (WBCs) of 9,375/μl, and proteinuria 3+. Biomarkers of renal tubular injury showed urinary N-acetyl-β-d glycosaminidase (NAG) 74.9 U/L (normal range 0.3–12 U/L), urinary neutrophil gelatinase-associated lipocalin (NGAL) 537.6 U/L (normal range 0.9–82 U/L), urinary albumin/creatinine 1,731.5 mg/g, urinary transferrin 1.74 mg/dl (normal range 0–0.2 mg/dl), and α1-microglobulin 0.95 mg/dl (normal range 0–1.25 mg/dl). Blood routine test showed that transient eosinophilia increased. Blood tests showed WBCs of 4,500/μl (neutrophils 35.6%, eosinophils 17%), lactate dehydrogenase 218 U/L, serum creatinine (sCr) 167 μmol/l (baseline sCr 81.7 μmol/l), erythrocyte sedimentation rate (ESR) 41 mm/h, and C-reactive protein <0.5 mg/L. The autoimmune antibody profile, immunoglobulin profile, and complement level were within normal range. Serum IgG4 was 0.497 g/l (normal range 0.03–2.01g/l). Fungal D-glucan test was negative. Serum procalcitonin was negative. Serum T-spot and urinary *Mycobacterium tuberculosis* were negative. Antibiotics were given, yet urinary symptoms were not relieved. His sCr level was significantly elevated to 211 μmol/l (baseline sCr 81.7 μmol/l). Blood tests showed that C-reactive protein was 41.7 mg/L; ESR was 120 mm/h. Repeated urine cultures were negative. Bilateral ureteral stenting was performed, and cystoscopy revealed diffused redness of the bladder mucosa, no sign of carcinoma infiltration. The pathologic change of the bladder tissue showed effacement of the bladder urothelium, hyperplastic granulation tissue, and infiltration of monocytes, lymphocytes, plasmacytes, and neutrophils in the bladder tissue. Immunohistochemistry staining of the bladder tissue showed positive staining of CD3, CD8, CD20, and CD117, yet negative staining of CD68, TIA-1, and PD-L1 in focal lesions (Figures 1A–F). The metagenomic next-generation sequencing (mNGS) of urine and ureter-bladder tissues was negative. Urinary ultrasonography and computed tomography showed mild hydronephrosis, dilated ureter, and thickened bladder wall (Figures 2A–C). Bladder residual urine was negative. The patient was eventually diagnosed as having immune-related ureteritis and cystitis on the 13th week after the occurrence of urinary symptoms. Methylprednisolone was administered at 60 mg (body weight 50 kg, equivalent to 1.5 mg/kg/day of prednisone) intravenously, and urinary irritation symptoms were relieved quickly after 3 days of treatment. After 2 weeks of methylprednisolone, sCr returned to the baseline level, and urinary WBC/RBC turned negative after 3 weeks of glucocorticoid therapy. Methylprednisolone was tapered gradually. Since the patient had a recurrence of urinary irritation symptoms, gross hematuria, and sterile pyuria on 16 September, methylprednisolone was increased to 40 mg intravenously, and the urinary irritation symptoms were alleviated quickly and urinary analysis improved. Bilateral ureteral stents were removed on 10 October 2022. The current dose of prednisone tablets is 25 mg. His urinary analysis showed that WBC was 145/μl, RBC was 5/μl, and sCr was 89.7μmol/l on 17 October 2022. The timeline of the treatment course was summarized in Figure 3.

TABLE 1 Summary of 10 cases of immune checkpoint inhibitor-related cystitis.

Case No.	Age/Gender/ Carcinoma	Immune checkpoint inhibitors	Pathology of urothelium	Dose of gluco-corticoid	Treatment outcome	Courses of glucocorticoid
1 (7)	61-year-old man metastatic melanoma	four cycles of nivolumab (every 15 days)	Infiltration of CD3+ and CD20 + and PD1+ lymphocytes	Prednisolone 0.5 mg/ kg/day	Improved after 7 days without relapse	Tapered after 1 month, weaned within 3 months
2 (8)	51-year-old man small cell lung cancer (SCLC)	five cycles of nivolumab (every 3 weeks)	Infiltration of CD3+ and CD8+ lymphocytes	Methylprednisolone 80 mg twice daily	Symptoms resolved after 3 days	Tapered over 6 weeks
3 (9)	67-year-old woman breast cancer (cT4bN1M1, Stage IV)	on day 97 after atezolizumab	Infiltration of CD8+ and intracellular antigen 1 (TIA-1) + lymphocytes in the urothelium eosinophilic infiltrations PD-L1 expression	Prednisolone 40 mg/ day (1 mg/kg/day)	Improved 2 days after therapy	Tapered after 4 days
4 (10)	53-year-old man pulmonary adenocarcinoma (cT1cN3M1c, Stage IVB)	on the fifth day after the third course of sintilimab	Undone	Methylprednisolone (80 mg, 1 mg/kg/ day)	Resolved after 17 days of corticosteroid treatment.	Eight weeks
5 (11)	50-year-old man lung squamous cell carcinoma (Stage IV)	seven cycles of nivolumab	Undone	Prednisolone 60 mg/ day (1 mg/kg/day)	Alleviated after 3weeks	Unknown
6 (11)	60-year-old man lung squamous cell carcinoma (Stage IV)	12 courses of nivolumab administration	Undone	Not used	Resolved after nivolumab withdrawal	Unknown
7 (12)	47-year-old man pulmonary adenocarcinoma (Stage IV)	18 cycles of nivolumab	Infiltration of eosinophils and plasma cells	Not used	Alleviated after the bladder biopsy	Unknown
8 (13)	78-year-old woman lung adenocarcinoma (cT4bN3M1a)	six cycles of pembrolizumab	Infiltration of CD8, TIA-1-positive lymphocytes and positive PD-L1 expression in the urothelium	Prednisolone 25 mg/ day	Alleviated after 19 days of treatment with steroid	Tapered within 2 months.
9 (14)	62-year-old man pulmonary squamous cell carcinoma (T4N0M1a, Stage IV)	On the 22nd day after administration of nivolumab	Mucosal epithelium completely sloughed off, interstitial edematous changes, slight lymphocytic infiltration	Steroid pulse therapy (methylprednisolone 500 mg × 3 days)	Resolved quickly	Maintenance dose 0.5 mg/kg/day, decreased gradually
10 (15)	48-year-old man intrahepatic cholangiocarcinoma (ICC)	Three cycles of nivolumab	Undone	Not used	Relieved after 3 months of drug withdrawal	Unknown
10 (16)	Case 10	Reoccurrence after three cycles of atezolizumab	Chronic inflammation of mucosal tissue, mucosal erosion, proliferation of granulation tissues and fibroblasts	Steroid hormones were given, which started at 2 mg/kg/ day	Improved quickly	Unknown

TIA-1 (T cell intracellular antigen 1 or cytotoxic granule-associated RNA-binding protein); PD-L1, programmed death ligand-1.

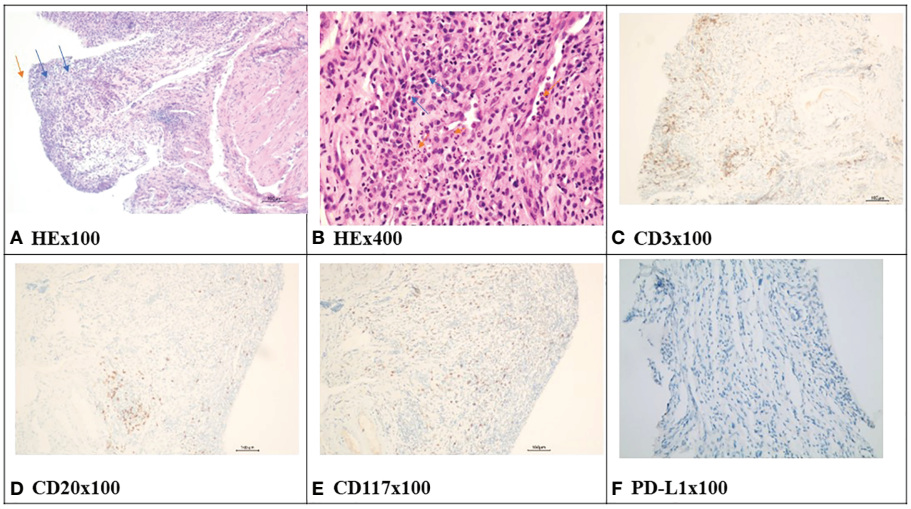

FIGURE 1
The HE and immunohistochemistry staining of bladder tissue in case 1. **(A)** showed effacement of the bladder urothelium (orange arrow), hyperplastic granulation tissue (blue arrow). **(B)** showed infiltration of monocytes, lymphocytes, plasmacytes (blue arrow), and neutrophils in the bladder tissue (orange arrow). **(C)** showed infiltration of CD3-positive lymphocytes in the bladder tissue (positive staining is brown). **(D)** showed positive infiltration of CD20-positive lymphocytes in the bladder tissue (positive staining is brown). **(E)** showed positive infiltration of CD117-positive mast cells in the bladder tissue (positive staining is brown). **(F)** showed that programmed death ligand-1(PD-L1) staining is negative in the bladder tissue. HE, hematoxylin-eosin.

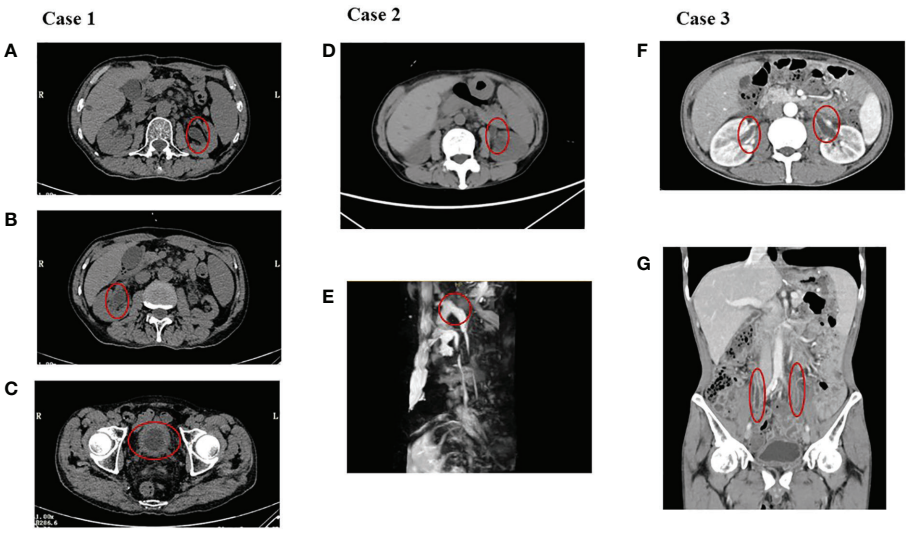

FIGURE 2
The images of the three cases. **(A–C)** showed hydronephrosis, dilation of the ureters, and a thickened bladder wall in case 1. **(D, E)** showed hydronephrosis and dilation of the ureter on the left in case 2. **(F, G)** showed dilation of the ureters and a thickened ureter wall in case 3.

Case 2

A 62-year-old woman was diagnosed as having Stage IV gastric carcinoma in July 2020. From July 2020 to December 2020, oxaliplatin 150 mg plus tegafur-gimeracil-oteracil potassium capsules 40 mg (days 1–14) were administered for six courses, then tegafur-gimeracil-oteracil potassium capsules 40 mg days 1–14 were given alone in January 2021. Because of

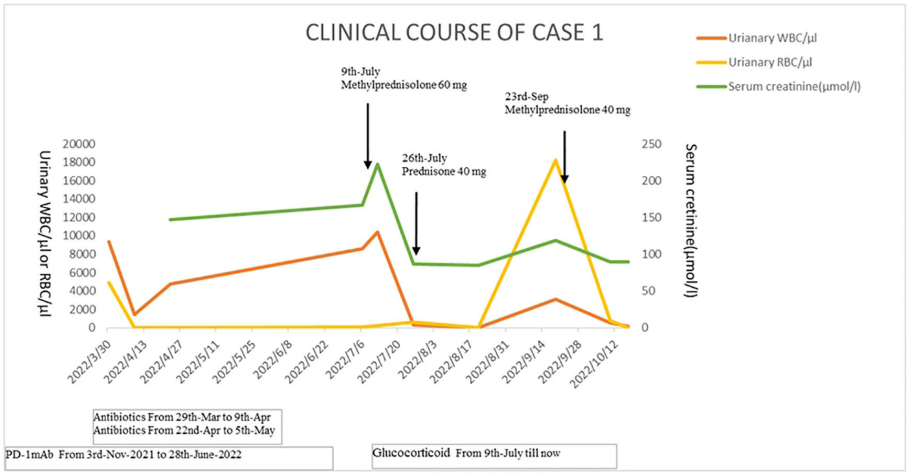

FIGURE 3
The timeline of the treatment course of case 1.

lymph node metastasis in the left clavicular and mediastinal area, she was given a regimen including sintilimab (PD-1 mAb) 200 mg, paclitaxel 200 mg (days 1–8), and tegafur-gimeracil-oteracil potassium capsules 40 mg (days 1–14) on 28 October 2022. Then, oxaliplatin 130 mg plus sintilimab (PD-1 mAb) 200 mg were given on 9 May 2022 and 1 June 2022. She did not have a medical history of hypertension, diabetes, or kidney disease.

She suffered from sudden-onset urinary irritation after three cycles of sintilimab (PD-1 mAb) treatment. Physical examination: T (Temperature) 36.3°C, P (Pulse) 70 bpm (beats per minute), R (Respiration) 16 bpm (breaths per minute), BP (Blood pressure) 110/60 mmHg. The abdomen was soft, no tenderness and rebound. Her renal percussive pain was negative. There was no edema in the lower limbs.

Urinalysis showed RBCs of 42/μl, WBCs of 17,916/μl, and proteinuria 3+. Blood tests showed WBCs of 2,300/μl (neutrophils 60.9%, eosinophils 4.3%) and C-reactive protein <0.5 mg/L. Her sCr was 56.2μmol/l (baseline sCr 56.4μmol/l). Serum T-spot and urinary *Mycobacterium tuberculosis* were negative. Repeated urine cultures were negative. Serum procalcitonin was negative. Biomarkers of renal tubular injury showed urinary NAG 29.2 U/L (0.3–12 U/L), urinary NGAL 247.6 U/L (normal range 0.9–82 U/L), urinary albumin/creatinine 466.9 mg/g, urinary transferrin 8.13 mg/dl (0–0.2 mg/dl), and α1-microglobulin 0.803 mg/dl (0–1.25 mg/dl). His 24-h urinary protein is 2.94 g. Urinary ultrasonography and CT showed mild hydronephrosis and dilation of the ureter on the left and a thickened bladder wall (Figures 2D, E). Bladder residual urine was negative. Antibiotics were given, and urinary symptoms were not relieved. The patient was eventually diagnosed as having immune-related ureteritis and cystitis. Methylprednisolone was given at 60 mg (body weight 44 kg, equivalent to 1.7 mg/kg/day of prednisone) intravenously after 18 days of urinary symptoms. After a week of methylprednisolone treatment, urinary symptoms were relieved and urinalysis was normal. The tapered prednisone was given at 45 mg and reduced by 5 mg per week. However, the patient discontinued prednisone by herself—assertion on 18 July 2022. And the urinary irritation symptoms and increased urinary WBC/RBC reoccurred on 21 July 2022. Prednisone was restarted at 30 mg/day and tapered gradually by 5 mg per week. Prednisone was discontinued on 26 September 2022. The last follow-up time was 10 October; urinary analysis remained negative and sCr was 55.5μmol/l. The timeline of the treatment course was summarized in Figure 4.

Case 3

A 49-year-old man was diagnosed as having gastric carcinoma (PT2N1M0, Stage IV) in September 2021. Subtotal gastrectomy was performed in September 2021, then tegafur-gimeracil-oteracil potassium capsules were administered for six courses. Nivolumab 300 mg was given on 28 March 2022 and 18 April 2022. He did not have a medical history of hypertension, diabetes, or kidney disease.

Symptoms with hematuria, pollakiuria, painful micturition, and fever developed on the second day after the second course of nivolumab therapy. Physical examination: T (Temperature) 38° C, P(Pulse) 100 bpm (beats per minute), R (Respiration) 18 bpm (breaths per minute), BP (Blood pressure) 100/65 mmHg. The abdomen was soft, no tenderness and rebound. His bilateral renal percussive pain was positive. There was no edema in the lower limbs.

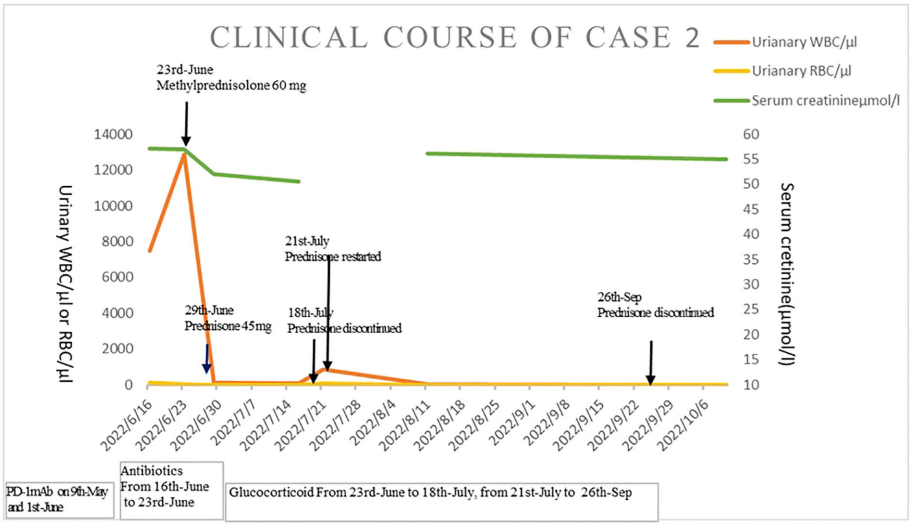

FIGURE 4
The timeline of the treatment course of case 2.

Urinalysis showed RBCs of 13,298/μl, WBCs of 2,506/μl, and proteinuria 3+. Urinary NGAL is 54.6 μ/l. Blood tests showed WBCs of 9,300/μl (neutrophils 72.8%, eosinophils 1.3%), C-reactive protein of 35.5 mg/L, lactate dehydrogenase of 164 U/L, sCr of 102 μmol/l, and ESR of 21 mm/h. Antibiotics were given. However, his sCr level significantly elevated to 190 μmol/l (baseline sCr 50.9 μmol/l). The autoimmune antibody profile, immunoglobulin profile, and complement level were within normal range. Fungal D-glucan test and limulus test were negative. Serum procalcitonin was 0.13 ng/ml. Serum T-spot and urinary *Mycobacterium tuberculosis* were negative. Blood culture was negative. Repeated urine cultures were negative. Urinary ultrasonography and CT showed mild hydronephrosis, dilated ureters, and thickened bladder wall (Figures 2F, G). Antibiotics were given, and urinary symptoms were not relieved. The patient was eventually diagnosed as having immune-related ureteritis and cystitis at the third week of urinary symptoms. Methylprednisolone was administered at 60 mg (body weight 44.5 kg, equivalent to 1.7 mg/kg/day of prednisone) intravenously, and body temperature returned to normal quickly and urinary irritation was relieved after 3 days of therapy. After 4 weeks of methylprednisolone, sCr returned to the baseline level and urinary WBC/RBC turned negative. The patient, who wished to initiate antitumor therapy earlier, complicated by intestinal fungal infections and steroid-induced diabetes during steroid treatment, reduced the dose of methylprednisolone quickly and discontinued it on 25 August 2022 by himself—assertion. Then, the patient had a recurrence of urinary irritation symptoms, gross hematuria, and sterile pyuria on 29 August. The methylprednisolone tablets were restarted at 16 mg, and the urinary irritation symptoms were alleviated quickly and urinary WBC/RBC returned to negative. The current dose of methylprednisolone tablets is 10 mg. Urinary WBC/RBC was negative from 26 September 2022 until now. His sCr was 60 μmol/l on 17 October 2022. The timeline of the treatment course was summarized in Figure 5.

Discussion

We reported three cases of PD-1 mAb-related ureteritis and cystitis that were diagnosed as esophageal carcinoma (n = 1) or gastric carcinoma (n = 2). All three cases manifested significantly increased urinary WBC/RBC, and two cases had gross hematuria. CT scan of the three cases showed dilation of the ureters and hydronephrosis. Obstructive nephropathy caused by renal calculus, carcinoma infiltration, and tuberculosis were excluded through CT scan, and repeated urine cultures were negative. The detection of mNGS in urine and bladder tissue was also performed in case 1, and the mNGS reports were negative.

Two of the three cases were complicated by acute kidney injury after two or six cycles of PD-1 mAb infusion, whose renal function returned to baseline level gradually after steroid therapy. Although we did not perform renal biopsy, we speculate that their renal pathology was probably acute interstitial nephritis according to their good responses to glucocorticoid therapy (3–5).

During follow-up periods, the three cases all showed reoccurrence of sterile pyuria during glucocorticoid decrement. The rapid reduction of steroid might be the main cause of

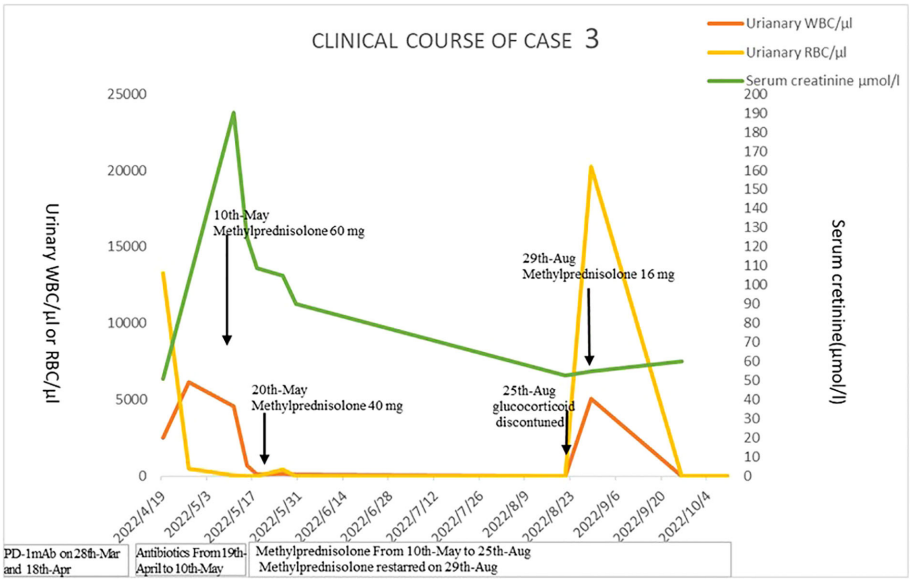

FIGURE 5
The timeline of the treatment course of case 3.

reoccurrence in both cases 2 and 3. However, severe pathologic lesions and more immune cell infiltration in bladder tissue might be due to four courses of PD-1 mAb infusion after the occurrence of immune-related ureteritis/cystitis in case 1. Moreover, ureteral stenting might aggravate the inflammation and edema of the urinary tract. Correspondingly, the patient's urinary symptoms and urinary analysis improved quickly after the upregulation of glucocorticoid dose and removal of ureteral stents. Longer sessions of glucocorticoids are speculated to improve the immunologic urinary injury in case 1.

We further performed a review of the literature about ICI-related ureteritis and cystitis (7–15). Eight papers were retrieved to be reports about ICI-related cystitis and one paper about ICI-related ureteritis/cystitis (10).

Ten cases were reported to have 11 episodes of urinary irritation after ICI therapy. Of the 10 cases, acute kidney injury was complicated in one case, and immune-related cystitis reoccurred in one case after PD-1 mAb restarting (7–15).

The ICIs, which could induce immune-related cystitis, were mainly PD-1/PD-L1 blockers (n = 10), including nivolumab (PD-1 mAb, n = 7) (7, 8, 11, 12, 14, 15), pembrolizumab (PD-1 mAb, n = 1) (13), sintilimab (PD-1 mAb, n = 1) (10), and atezolizumab (PD-L1 mAb, n = 2) (9, 15). The three cases in our report were also diagnosed as PD-1 mAb-related ureteritis and cystitis. It seems that PD-1/PD-L1 pathway blockage might induce immune-related ureteritis and cystitis more commonly.

The expression of PD-L1 in bladder tissue was identified in patients with severe bladder inflammation (16). It is speculated that cytotoxic T-cell activation induced by PD-1/PD-L1 mAb might attack both carcinoma and normal urothelium with PD-L1 expression (9, 13). In case 1, there was CD3 (7, 8, 13), CD8 (8, 9, 13), and CD20 (7) positive expression in bladder tissue, as reported by previous case reports of immune-related cystitis. CD117-positive mast cells were identified in a focal lesion, which means that mast cells are also involved in the immune-related cystitis in case 1. We also performed immunohistochemistry staining of TIA-1, CD68, and PD-L1, and the results were negative. Consistent with the report by Zhu et al. (8), the negative expression of PD-L1 was detected in the urothelium of case 1. It might be due to the severe injury and shedding of the bladder urothelium.

Immune-related ureteritis and cystitis were also observed in autoimmune disease, including lupus, Sjögren's syndrome, and vasculitis (17, 18), which suggested that the immune attack of PD-L1 is not the only immunopathogenesis of immune-related ureteritis/cystitis. The exact mechanism of immune-related ureteritis/cystitis needs further research. Biomarkers of ICI-related ureteritis/cystitis with high specificity are urgently needed.

IrAEs have been suggested to occur at any time but usually develop within the first few weeks to months after administration initiation (19). In previous case reports of ICI-related cystitis, the time from the initiation of therapy to the development of cystitis ranged from 2 to 12 months (the third to 18th cycles of ICIs) (7–15). In our report, the patients presented with urinary symptoms that ranged from 3 weeks to 15 weeks

(the second to sixth cycles of PD-1 mAb therapy). Hence, it is vital to monitor the possibility of immune-related ureteritis/cystitis if there are any new-onset urinary symptoms and abnormal urinary examination during ICI therapy. In our report, the three cases did not manifest other irAEs including skin, endocrine, pulmonary, and gastrointestinal injury. It makes the early diagnosis of urinary irAEs a challenge. Therefore, urinalysis, renal function, and urinary imaging examination are recommended to be monitored regularly during ICI therapy. It contributes to identify immune-related ureteritis and cystitis earlier to avoid the abuse of antibiotics and occurrence of corresponding adverse effects.

In previous case reports of ICI-related ureteritis/cystitis, nine cases received glucocorticoid therapy, seven cases received corticosteroids at 1.7 mg/kg/day (8–11, 15), two cases received glucocorticoids at 0.5 mg/kg/day (7, 13), and one case received glucocorticoid pulse therapy (14). All cases acquired remission of urinary symptoms and normal urinalysis results. The glucocorticoid was tapered gradually within 2 months in most cases.

Since all three cases in our report presented with severe urinary irritation symptoms, and two cases were also complicated by acute kidney injury, the glucocorticoid therapy (methylprednisolone) was started at a dose of 60 mg/day^{-1} (1.5–2.0 mg/kg/day). Urethral pain and pollakiuria disappeared several days after initiating steroid therapy. Urinary WBC/RBC returned to negative within 1–4 weeks. Renal function returned to baseline level within about 1 month in cases 1 and 3. Rapid tapering of glucocorticoid dose might lead to the reoccurrence of urinary irAEs. The three cases in our report were recommended not to receive ICI treatment again.

According to the published literature and the experience acquired in our cases, the initial dose and treatment course of glucocorticoids can be evaluated according to the following points: ① the grade of urinary symptoms (19, 20); ② whether complicated with ICI-related acute interstitial nephritis or glomerulonephritis (20); ③ whether complicated by irAEs in other organs (20). The reoccurrence of immune-related ureteritis/cystitis might occur during rapid tapering of glucocorticoids.

Conclusions

ICI-related ureteritis/cystitis is rarely reported, and the diagnosis poses a challenge. It is vital to monitor any new-onset urinary symptoms and abnormal urinalysis during ICI therapy to identify immune-related ureteritis/cystitis earlier. Urinalysis, renal function, and urinary imaging examination are recommended to be performed regularly during ICI therapy courses. It contributes to identify immune-related ureteritis/cystitis earlier to efficiently alleviate immunologic urinary tract injury through glucocorticoid therapy and avoid the abuse of antibiotics. It is necessary to follow patients closely during steroid decrement to make prompt treatment of reoccurrence.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

Written informed consent was obtained from the individual (s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

JL (First author and Corresponding author) contribute to do the design, clinical data collection, and write the manuscript. Y-FY (co-first author), contribute to revise the manuscript, equal contribution and share first authorship. X-WQ contributes to do immunohistochemistry staining of bladder tissue, read and evaluate the pathologic change of the case reported in our paper. YD and C-QL contribute to collect the clinical data of the patients. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Advances in CT features and radiomics of checkpoint inhibitor-related pneumonitis: A short review

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Checkpoint inhibitor-related pneumonitis (CIP) is a complication of immunotherapy for malignant tumors that severely limits the treatment cycles as well as endangers patients' health. The chest CT imaging features or typing of CIP and the application of radiomics will contribute to the precise prevention, early diagnosis and instant treatment of CIP. This article reviews the advances in the CT features and the application of radiomics in CIP.

KEYWORDS

checkpoint inhibitor-related pneumonitis (CIP), computed tomography (CT), radiomics, radiation-induced pneumonitis (RIP), cancer

Introduction

Programmed death 1 (PD1)/programmed death-ligand 1 (PD-L1) targeted immune checkpoint inhibitors (ICIs) have revolutionized cancer treatment. With the continuous publication of results from large randomized controlled clinical studies, ICIs alone or in combination with radiotherapy, chemotherapy, or anti-angiogenic therapy are becoming the first-line treatment of choice for most major cancer types (1–4). However, the incidence of immunotherapy-related adverse effects (irAEs) is inevitable and relatively high. For example, a meta-analysis with a total of 1063 Chinese patients in 13 clinical studies enrolled, of whom 922 (86.7%) received ICIs monotherapy and 141 (13.3%) received ICIs plus chemotherapy or anti-angiogenesis, reported that the incidence of irAEs of any grade was 43.3%, and 4.3% of patients discontinued the treatment due to the severe irAEs (5). The incidence of checkpoint inhibitor-related pneumonitis (CIP) was reported to range from 1%-4% with single-agent immunotherapy (6) and up to 6.6% with combined strategies (7). Early clinical symptoms are not easy to detect, and severe grade 3-5 CIP may lead to severe respiratory failure and is one of the major fatal adverse reactions, so it is urgent to identify or predict the occurrence of immunotherapy-related pneumonitis accurately.

CIP can be diagnosed by meeting the following three criteria: (i) history of ICI medication; (ii) newly appeared lung shadow; (iii) excluding lung infection, lung tumor progression, other causes of interstitial lung disease, pulmonary embolism, pulmonary

vasculitis and pulmonary edema. However, during the treatment of tumor patients, other factors such as infections, radiation therapy, other drugs such as targeted drugs, chemotherapy, etc. are often mixed, thus it is often difficult to accurately diagnose CIP and select the appropriate treatment plan accordingly timely. Although some biomarkers such as interleukin (IL) including IL2 and IL17a, as well as circulating CD8+ T-cells and neutrophil/lymphocyte ratio have been found to correlate with the incidence of CIP (8). It has also been suggested that chronic lung disease such as chronic obstructive pulmonary disease, emphysema, and interstitial lung disease and history of prior chest radiation therapy may be independent risk factors for the occurrence of CIP, but there are no clear, stable, and mature identification or prediction models that can be applied in the clinical setting.

Radiomics is an emerging technology in medical imaging with the automated extraction of multidimensional imaging data from medical images aiming for comprehensive visualization and characterization of the disease-involved tissue and its microenvironment. Radiomics-based approaches that quantitatively identify associations between the extracted imaging data and clinical characteristics or outcomes and use these associations to construct predictive models thereby providing a solution to clinical problems, such as those that have been developed for identification of benign or malignant small pulmonary nodules, interpretation of COVID-19 pneumonia, and other various aspects of the medical field. While there are many studies on the use of radiomics to predict the efficacy of immunotherapy, there is no systematic review of the emerging field of radiomics to identify or predict the occurrence of CIP, although several studies were published in recent years. Therefore, we attempt to review the relevant content and provide some insights.

CT imaging patterns of CIP

Computed tomography (CT) is an important imaging modality for the diagnosis of lung disease, and the imaging features on CT of the lung are crucial to diagnose CIP correctly. American Thoracic Society/European Respiratory Society (ATS/ERS) revised and supplemented the international consensus on the classification and diagnostic criteria of interstitial pneumonia in 2013 (9), and subsequent scholars have mostly based their phenotypic patterns of CIP images on these criteria, which include organizing pneumonia (OP), nonspecific interstitial pneumonia (NSIP), hypersensitivity pneumonitis (HP), bronchiectasis, and acute interstitial pneumonia - acute respiratory distress syndrome (AIP-ARDS).

In 2016, Naidoo et al. first classified the following imaging subtypes based on CT in 27 patients with CIP: cryptogenic organizing pneumonia (COP) (2/27, 19%), ground glass opacity (GGO) (10/27, 37%), NSIP (2/27, 7%) and HP (6/27, 22%), and not otherwise specified (NOS) pneumonia (4/27, 15%), and found that CT imaging patterns were essentially consistent throughout the patients' clinical course, except for two patients (10). Lin et al. found that predominant patterns were GGO (43.6%), NSIP (25.5%), COP (18.2%), and follower by NOS (12.7%) based on a similar classification pattern, and AIP-ARDS indicated severe pneumonia (correlation coefficient = 0.707, $p < 0.001$) (11). However, the above study analyzed the radiographic element GGO together with the imaging patterns, which was not appropriate. According to the diagnostic criteria for interstitial pneumonia published by ATS/ERS, Delaunay et al. instead analyzed in a larger sample size (64 cases) and found COP to be the most common pattern (15/64, 23.4%), followed by HP (10/64, 15.6%), NSIP and bronchiectasis in 7.8% (5/64) and 6.3% (4/64), respectively and another 23 with unspecified pneumonia (35.9%) (12). Subsequent published studies consistently found the COP pattern as the most common type in both lung and non-lung tumors (13–15). There was no significant difference in imaging patterns between early-onset and late-onset CIP (15). The AIP/ARDS type turned out to have the highest adverse effect grade, while NSIP and HP seemed to be mild (median grade: 3, 2, 1, 1; $p = 0.006$) (13).

Specifically looking at the CT radiographic elements, GGO was the most common feature (52/64, 81.3%), followed by consolidation (34/64, 53.1%), bronchiectasis (11/64, 17.2%), interlobular septal thickening (10/64, 15.6%) and intralobular lines (14/64, 21.9%), etc. and mainly showed diffuse lung involvement (12). Consolidation was common in patients with lung cancer and less frequent in non-lung cancer patients (29%), while nodular lesions were found in only a minority of patients in the non-lung cancer group (29%) (14). Interestingly, Balaji et al. specifically looked at the steroid-refractory CIPs, and reported a similar pattern, with GGOs (50%, 6/12) as the predominant ones and mostly involving bilateral lung fields (75%, 9/12) (16). Similarly, Imran et al. reported a few cases of rapid deterioration even after adequate treatment with CT features of diffuse GGOs (17), however, the imaging features are similar in common CIPs without novel findings, which should be further explored and well organized.

Identification of CIP by CT radiomics

Radiation therapy is often involved and plays an important role in the immunotherapy process, improving local control of the tumor as well as acting as an immune adjuvant to sensitize the efficacy of immunotherapy. However, in the case of non-infectious pneumonia, the attribution of pneumonia by radiation or immunotherapy is often difficult for clinicians to distinguish, thereby making it difficult to treat. Hence, using radiomics to analyze the differences in imaging features between radiation induced pneumonitis (RIP) and CIP and to classify and identify them will help clinical decision making and benefit patients.

The first systematic comparison of CT features of RIP and CIP was performed by Chen et al. at Johns Hopkins University, which included 82 patients: 30 after RT+ICI, 29 after thoracic RT, and 23 after ICI. Compared with RIP, CIP was more likely to be bilateral (65% vs. 28%; p = 0.01), involve more lobes (66% vs. 45%), and was less likely to have sharp borders (17% vs. 59%; p = 0.004). The area under curve (AUC) of the machine learning model to differentiate CIP and RIP reached 0.76 based on the following 7 imaging features: bilateral, number of lobes, volume of lung involved, multifocal, radiographic elements, radiographic patterns, and sharp border (18). A similar radiomics study done by Cheng et al. in China, developed the linear SVM classification model based on three radiomics features (intensity histogram, bag-of-words [BoW] features, and gray-level co-occurrence matrix [GLCM]), and a 10-

fold cross-validation in patients receiving only ICI or RT showed robust results with AUCs of 0.937. The model was then tested in patients receiving ICI+RT and could achieve an AUC of 0.896 (19). The retrospective study by Qiu et al. included a larger sample (126 cases) and finally identified the Rad-score (11 imaging histological features) with the potential to distinguish between CIP and RIP, and also found that bilateral involvement and sharp border were associated with the distinguishment of CIP and RIP. Combining the Rad-score and the above two features, authors created a robust model showing good performance in both the training dataset (empirical-based AUCs of 0.953) and the validation dataset (AUC = 0.947) (20), which is also the model with the best recognition performance reported in the literature so far.

These results suggest that CT-based radiomics has good potential in differentiating CIP from RIP in lung cancer and may become a practical tool in the future to provide a valuable differential diagnosis for the attribution of pneumonia in patients treated with concomitant ICI and RT.

Prediction of CIP by CT radiomics

Treatment after the onset of CIP is often difficult to ensure patients' quality of life and long progression-free survival because of the severity of the disease, making it a hot issue to predict the possible onset of CIP well before ICI treatment and to perform primary prevention or to screen out the optimal population. The earliest exploration was conducted by Colen et al. at MD Anderson, who analyzed a total of 1860 imaging features on baseline chest CT from 2 patients who developed CIP and 30 patients who did not develop CIP. Using feature selection methods of maximum correlation and minimum redundancy, abnormality detection algorithms, and leave-out cross-validation, 2 radiological features with significant differences were finally identified: skewness (a measure of histogram symmetry) and angular variance of the sum of squares (a measure of dispersion) (21). Spiele et al. from the University of Miami, on the other hand, performed a proof of concept for the prediction of pneumonia after radiation therapy combined with immunotherapy in a mouse Lewis lung cancer model. Mice were bilaterally imaged with CT and MRI after subcutaneous tumor formation and blood collection, and then treated with RT of the right abdominal tumor only (3*8Gy) followed by intraperitoneal injection of PD-1 inhibitors. They found that 3 CT radiomic features (mean grayscale, histogram kurtosis and co-occurrence matrix entropy) and 1 MRI feature (histogram kurtosis) together with baseline neutrophil-to-lymphocyte ratio (NLR) and granulocyte-macrophage colony-stimulating factor (GMSF) levels were positively correlated with CD45 infiltration (22). However, it is important to note that this model only assessed the CD45 infiltration levels to indicate the occurrence of pneumonia is not sufficiently reasonable and could not distinguish between RIP and CIP. Recently, Tan et al. retrospectively collected baseline CT images and clinical data from 24 patients who experienced CIP after immunotherapy and 24 controls who did not experience CIP. The model was pre-trained using a two-stage migration learning on a large natural image dataset and a large CT image dataset of pneumonia, then finally trained on locally collected CT image data. Finally, contrast learning was used to mine high-performance imaging feature models. Using five-fold cross-validation, the model was able to accurately predict CIP patients and non-ICIP patients with an AUC of 0.918 and an accuracy of 0.920 (23). This study strongly indicates that deep learning has great potential for identifying patients at risk of developing CIP.

From these studies, we could conclude that the prediction models proposed by big data-based radiomics studies may lead to effective risk stratification, close monitoring and timely management of CIP in the future to improve treatment outcomes. However, given the complexed clinical course of CIP, above radiomics studies did not show the exact prediction ability of those >3 grade CIPs or those rapid deteriorating CIPs that warrant emergent treatments.

Shortcomings and challenges of radiomics to predict or identify CIP

The application of radiomics in CIP has attracted some scientific interest, and in general, some excellent findings on the prediction and identification of CIP by radiomics have been reported. However, it has to be pointed out that there are still many problems from the mature development of the model and its real application in the clinic: (1) the image input, including the technical factors of CT imaging and the segmentation of region of interest (ROI). Although chest CT has been widely used in major hospitals, differences in hardware, scanning protocols and reconstruction algorithms of different manufacturers still have an impact on the extraction of image histological features. Secondly, the segmentation of ROI has been studied either artificially by experts or using software segmentation, but the repeatability of segmentation among different segmentation experts or even within software still needs to be improved (24, 25). (2) In terms of model maturation and validation, most of the current radiomics studies have small sample sizes, and more prospective studies with larger sample sizes are needed in the future to build models that can uncover more valuable radiomics features, and indeed more external data are needed to validate the accuracy of radiomics models. In addition, it is worth looking forward to whether the combination of radiomics with other available data such as pathology, genomic alterations or a variety of blood test results can bring more robustness and accuracy to the model. (3) As for the molecular biological significance of the model, for example, most of the radiomics features identified in the prediction study are pre-defined artificial features, and the potential molecular biological significance of these features needs to be further explored. However, because the mechanisms of CIP occurrence are still largely unclear, it is difficult to conduct relevant studies. In addition, radiomics studies of paired pre- and post-occurrence of CIP are rarely reported, and only correlational findings are shown, thus it is worthwhile to analyze whether causal findings can further improve the predictive performance. (4) For the prediction or identification of severe CIPs that need emergent treatment, and prediction of steroid-refractory or rapid progressing ones without

relief modalities, still no radiomics data are available and relevant studies are thereby recommended.

Author contributions

JH conceived the concept, created the draft. XC, BX and SM discussed and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Study protocol: a prospective single-center study for non-invasive biomonitoring of renal complications in cancer patients treated with immune checkpoint inhibitors

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Background: The advent of immune checkpoint inhibitors (ICIs) has powerfully broadened the scope of treatment options for malignancies with an ongoing increase of indications, but immune-related adverse events (irAEs) represent a serious threat to treatment success. Agents directed against programmed cell death protein 1 (PD-1) or its ligand 1 (PD-L1) are known to cause renal complications with an incidence of 3%. In contrast, subclinical renal involvement is estimated to be much higher, up to 29%. We recently reported about urinary flow cytometry-based detection of urinary PD-L1-positive (PD-L1$^+$) kidney cells correlating with tubular PD-L1-positivity that reflected susceptibility to develop ICI-related nephrotoxicity as an irAE attending ICI treatment. Therefore, we designed a study protocol to evaluate urinary detection of PD-L1$^+$ kidney cells as a tool for non-invasive biomonitoring of renal complications in cancer patients treated with ICIs.

Methods: A prospective, controlled, non-interventional, longitudinal, single-center observational study will be conducted at the Department of Nephrology and Rheumatology of the University Medical Center Göttingen, Germany. We intend to enroll approximately 200 patients treated with immunotherapy from the Departments of Urology, Dermatology, and Hematology and Medical Oncology of the University Medical Center Göttingen, Germany. First, we will assess clinical, laboratory, histopathological, and urinary parameters in addition to urinary cell collection. Then, we will perform a correlative analysis between urinary flow cytometry of different PD-L1$^+$ cell of renal origin with the onset of ICI-related nephrotoxicity.

Discussion: Because of growing ICI-treatment applicability with an expectable incidence of renal complications, providing cost-efficient and easily performable diagnostic tools for treatment-attendant and non-invasive biomonitoring becomes vital to improve both renal and overall survival rates in cancer patients receiving immunotherapy.

Trial registration: https://www.drks.de, DRKS-ID DRKS00030999.

KEYWORDS

PD-L1, AIN, irAE, ICI, urinary flow cytometry, TEC

Introduction

With the advent of immune-checkpoint inhibitors (ICIs), the scope of cancer-treatment options has seen a powerful increment, wherein reactivation of CD8-positive T-cell cytotoxicity constitutes its functional ground (1). Established neutralizing antibodies are directed against co-inhibitory auxiliary proteins expressed on tumor cells engaging in mechanisms of so-called immune evasion. Blockade of programmed cell-death protein 1 (PD-1) and its ligand 1 (PD-L1) is associated with remarkable therapy responses, especially for solid tumor entities featuring restricted therapeutic options. Still, immune-related adverse events (irAEs) pose a serious threat to treatment success, including maintenance of tumor remission (2–6). Of these irAEs, skin, gastrointestinal, hepatic, and endocrine adverse effects are the most common (7). Kidney involvement is known to occur with an incidence ranging from 2 to 3%; subclinical affection is estimated even higher, up to 29% (8–16). Glucocorticoid therapy and discontinuation of the causative agent are the only available measures with the side effect of delayed cancer treatment. Once affected, kidneys are more prone to a renal relapse after ICI re-exposition (9–11). Nephrotoxicity related to ICI therapy mainly consists of acute interstitial nephritis (AIN) and, to a lesser extent, glomerular nephropathies (8–19). This nephrotoxicity remains unclear and deserves to be characterized regarding clinical patterns and the underlying mechanisms.

We previously reported aberrant PD-L1 expression distinct to renal compartments in ICI-related nephrotoxicity, reflecting susceptibility to develop renal irAE (20, 21). PD-L1 positivity is different in intrarenal compartments and predominantly expressed in the tubuli, which correlates with elevated serum levels of C-reactive protein (CRP) as a non-specific marker of systemic inflammation (20). Moreover, PD-L1 expression was also observed in ICI-naïve renal pathologies implying PD-L1 upregulation as an indicator of ongoing kidney damage before this becomes clinically detectable by conventional methods (20). Interestingly, urinary flow cytometry-detected PD-L1-positive (PD-L1$^+$) kidney cells correlated with intrarenal PD-L1 positivity (20). Therefore, we designed a study protocol to evaluate urinary detection of PD-L1$^+$ kidney cells of different origins as a tool for non-invasive and therapy-attendant biomonitoring of renal complications in cancer patients receiving immunotherapy.

Methods

Study design and study population

Our observational single-center study is prospective, controlled, longitudinal, and non-interventional (trial registration: https://www.drks.de, DRKS-ID: DRKS00030999). It will be performed at the Department of Nephrology and Rheumatology at University Medical Center Göttingen (UMG), Germany. In addition, cancer patients receiving ICI treatment at the Departments of Dermatology, Urology, Hematology, and Medical Oncology of the UMG will be enrolled. The type and dosing schedule of the ICI therapy will be performed as indicated by the responsible medical specialist and carried out independently from the study investigations. A sample size of 200 patients was calculated based on the reported incidences of ICI-related nephrotoxicity.

Patient enrollment and study conduction

Inclusion criteria are patients 18 years or older, written informed consent, and intended immunotherapy. In addition, patients enrolled in other ongoing trials of immunotherapies with a blinded study design are considered ineligible. Within the framework of routine medical care of patients in the respective departments, eligible patients are identified and included after documented written consent. Figure 1 shows a STROBE flow chart of the study protocol featuring an overview of inclusion and exclusion criteria. After providing written consent, a baseline visit will be conducted, where baseline demographic data, medical history, medication, and clinical symptoms will be collected. Physical examination includes the assessment of height, weight, vital parameters, general condition, the status of the integument, neck with the thyroid gland, head, ears, eyes, throat, abdomen, limbs, joints, and auscultation of the heart and lungs. Laboratory data assessments include differential blood cell counts, serum levels of sodium, potassium, calcium, creatinine, blood urea nitrogen (BUN), uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase, bilirubin, lactate dehydrogenase (LDH), lipase, blood glucose, creatine kinase (CK), thyroid-stimulating hormone (TSH), T3, T4, and CRP. In addition, viral serology for the human

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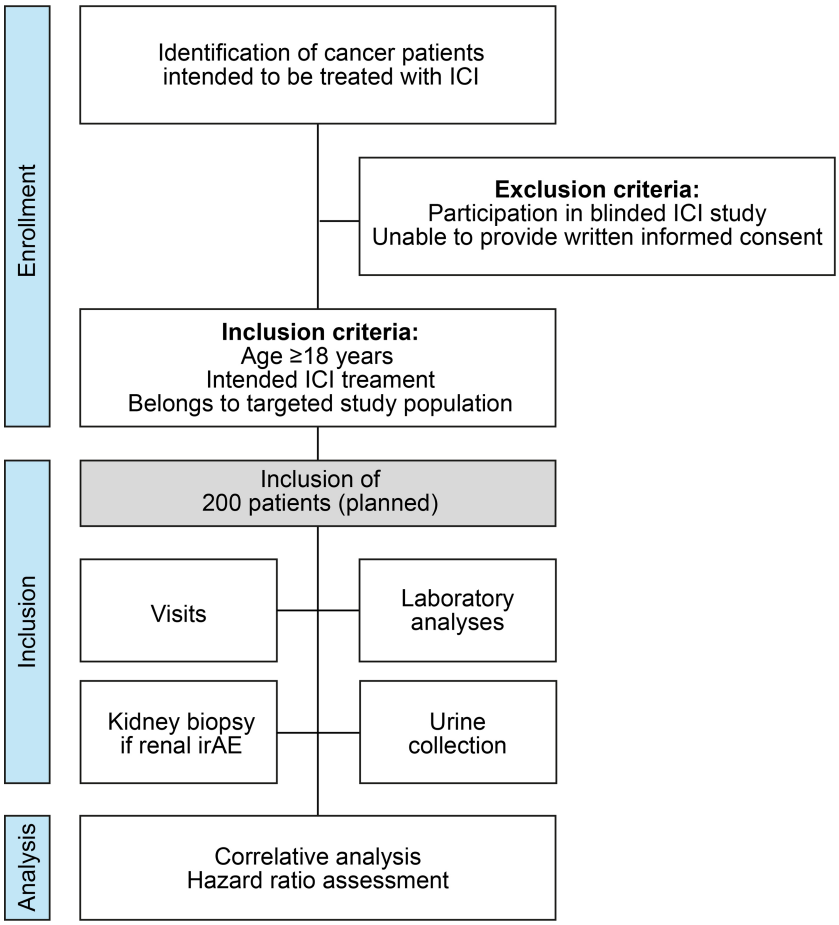

FIGURE 1
STROBE flow chart of the study protocol. ICI, immune checkpoint inhibitor; irAE, immune-related adverse event; STROBE, strengthening the reporting of observational studies in epidemiology.

immunodeficiency virus (HIV), hepatitis A, B, and C, cytomegalovirus (CMV), and the Epstein-Barr virus (EBV) will be tested at baseline. Moreover, autoantibody testing will be performed once at baseline and afterward as clinically indicated and includes antinuclear antibodies (ANA), anti-neutrophilic cytoplasmic antibodies (ANCA), extractable nuclear antigen (ENA), anti-double stranded desoxyribonucleic acid (anti-dsDNA), anti-cyclic citrullinated peptide (anti-CCP) and rheumatoid factor (RF). In addition, urinanalysis will be performed at each visit with urine dipstick and urine sediment, and an additional asservation of 100 mL of fresh urine samples will be performed. Follow-up visits are scheduled every 12 (–16) weeks; unscheduled visits will be performed if an irAE is suspected. The end-of-study visit will be conducted after 48-52 weeks. The schedule of visits and assessments is shown in Table 1.

Asservation of urine cells

Fresh urine samples will be collected (100 mL) and loaded into an automated cytospin machine (Shandon cytospin, Thermo Scientific, Pittsburgh, USA) following the manufacturer's instructions and centrifuged at 1000 revolutions per minute (rpm) for 10 minutes. The cell pellet is resuspended in a mixture of phosphate-buffered saline (PBS) and bovine serum albumin (BSA). Cell viability and quantity are measured in a cell counter. Urine cells are deep frozen in Gibco™ CTS™ Synth-a-Freeze™ medium at -80°C. Cells can be further processed for urinary flow cytometry.

Flow cytometry of urinary cells

Antibody staining of cells in suspension follows standard protocols. Thawed or freshly collected cells are centrifuged at 1000 rpm at 4°C for 8 minutes. The PD-L1$^+$ cell line KARPAS 299 will be used as a positive control and unstained cells as negative controls for the analysis of urinary cells. Cell suspensions are washed and resuspended in PBS and stained using antibodies against PD-L1 (APC anti-human CD274, B7-H1, PD-L1, clone 29E.2A3, Biolegend, San Diego, USA) in combination with the podocyte marker podocalyxin (anti-TRA-1-81-PE, human, clone REA246, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). Identification of tubular epithelial cell (TEC) will be done by cytokeratin (anti-cytokeratin-FITC, human, clone CK3-6H5, Miltenyi Biotec GmbH,

TABLE 1 Schedule of patient visits and assessments.

Week/occasion	0	12 (+4)	48 (+4)	Routine visits during ICI application	Non-scheduled visits (occurrence of irAE)
Written informed consent	X				
In-/exclusion criteria	X				
Enrollment	X				
Medical history	X	X	X	X	X
Physical examination[1]	X	X	X	X	X
Vital parameters[2]	X	X	X	X	X
Weight and height[3]	X	X	X	X	X
Laboratory analyses[4]	X	X	X	X	X
Urinary analyses[5]	X	X	X	X	X
Viral serologies[6]	X				
Antibody screening[7]	X				X
Medication	X	X	X	X	X
Assessment of irAE[8]	X	X	X	X	X

[1]Includes general condition, status of the integument, neck with thyroid, head, ears, eyes, throat, abdomen, limbs, joints and auscultation of heart and lungs, orienting neurological examination.
[2]Blood pressure and heart rate after five minutes of rest.
[3]Weight in kilogram, height in centimeters.
[4]Including differential blood cell count, serum levels of sodium, potassium, calcium, creatinine, BUN, uric acid, AST, ALT, gGT, bilirubin, LDH, blood glucose, CK, TSH, T3, T4, CRP.
[5]Urinary dipstick, urinary sediment, 100 mL fresh urine sample.
[6]HIV, hepatitis A/B/C, CMV, EBV.
[7]RF, anti-CCP, ANA, ENA, ANCA, anti-dsDNA.
[8]Documented on a separate data sheet.
ALT, alanin aminotransferase; ANA, antinuclear antibodies; ANCA, anti-neutrophilic cytoplasmic antibodies; Anti-CCP, cyclic citrullinated peptide; Anti-dsDNA, anti-double stranded desoxyribonucleic acid; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CK, creatine kinase; CMV, cytomegalovirus; CRP, C-reactive protein; EBV, Epstein-Barr virus; ENA, extractable nuclear antigen; gGT, gamma-glutamyl transferase; HIV, human immunodeficiency virus; ICI, immune checkpoint inhibitor; irAE, immune-related adverse event; LDH, lactate dehydrogenase; RF, rheumatoid factor; TSH, thyroid-stimulating hormone.

Bergisch Gladbach, Germany) with differentiation between proximal TECs by CD-10 (CD10-PE-Vio770, human, clone 97C5, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany), and distal TECs by EpCAM (CD326 (EpCAM)-APC-Vio770, human, clone HEA-125, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) (22). Cells will be incubated with antibody mixtures for 15 minutes at 4°C in the dark and gated according to positive and isotype-negative controls. For dead cell identification, propidium iodide (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) will be used to gate out non-viable cells. According to standard protocols, cells will be analyzed with the FACS Canto II machine of the cell sorting unit at the Department for Hematology and Medical Oncology of the UMG.

Residual material of renal biopsies

During clinical ICI treatment, the indication for renal biopsies in cases of kidney injury depends on the responsible nephrologist. Residual material of renal biopsies can be utilized for additional diagnostic procedures if ICI-related nephrotoxicity is suspected.

Immunofluorescence

For immunofluorescent stainings, primary antibodies against PD-L1 (ab205921, Abcam, Cambridge, UK) and Alexa Fluor 488 (Invitrogen, Carlsbad, CA) secondary antibodies will be used, nuclear staining will be performed using 4',6-diamidino-2-phenylindole (Vector Laboratories).

Immunohistochemistry

Formalin-fixed, paraffin-embedded kidney sections will be deparaffinized in xylene and rehydrated in ethanol containing distilled water. Tissue sections will be stained using antibodies against PD-L1 (ab205921, Abcam, Cambridge, UK), and labeling is performed using Novolink™ Polymer Detection System (Leica Biosystems, Wetzlar, Germany) according to the manufacturer's protocol. Nuclear counterstain will be performed using Mayer's Hematoxylin Solution (Sigma, St. Louis, USA).

Sample size calculation

Due to the explorative design of the current study evaluating potential parameters that affect the risk of irAEs, the sample size calculation is not hypothesis-driven. Based on the feasibility to enroll, analyze, and follow up, a total number of 200 patients was chosen. The aim of the current study is the hazard ratio assessment of different influencing factors on irAEs related to the kidneys.

Statistical methods

To assess putative correlations between compiled data, regression methods (Cox regression, logistic regression, Poisson regression) will be performed. Moreover, time-to-event analyses and Kaplan-Meier survival estimates will be conducted. Variables will be tested for normal distribution using the Shapiro-Wilk test. Non-normally distributed continuous variables will be expressed as the median and interquartile range (IQR), and categorical variables as frequency and percentage. Statistical comparisons are not formally powered or prespecified. The Mann-Whitney U-test will be used for group comparisons to determine differences between median values. Spearman correlations will be visualized by heatmaps reflecting mean values of Spearman's ρ. If not otherwise, specified all calculations are performed with GraphPad Prism (Version 9.5.0 for macOS, GraphPad Software, San Diego, California USA, "www.graphpad.com") or STATA (STATA/MP version 16.1 for Windows, Stata Corp LLC, College Station, TX, USA). Due to the non-interventional, observational, and prospective study design, hypothesis testing will not be conducted.

Discussion

Kidney affection occurring as an irAE during immunotherapy is a severe and potentially life-threatening complication endangering treatment success, hence the overall survival of cancer patients. Clinically apparent renal involvement is estimated to occur with an incidence of 3%. In contrast, previous investigations of biopsy-proven ICI-related nephrotoxicity reported a much higher frequency of up to 29% of cases implying that conventional diagnostic methods only detect advanced and pronounced kidney injury and are unable to detect early and milder forms of renal involvement (8–16). Renal biopsy is the gold standard for the etiological assignment of kidney affection but carries an increased risk of bleeding complications in the cancer-patient population. Moreover, repeated renal biopsies during a complete treatment course iteratively subject patients to the risk of hemorrhagic events. Despite discontinuing ICI treatment and steroid therapy, the kidneys stay endangered to relapse. In case of renal relapses due to cancer-treatment continuation, the clinical course may even be more fulminant, requiring kidney replacement therapy (KRT) (2). Thus, new tools for biomonitoring in clinical routine will be helpful for the early detection of complications.

Our study aims to evaluate the urinary detection of PD-L1$^+$ kidney cells based on urinary flow cytometry as a non-invasive biomonitoring tool for renal complications in cancer patients treated with immunotherapy. The concept of urinary flow cytometry-based detection of renal and immune cells reflecting so-called "biosignatures" was first described for renal allograft pathology (22, 23). Adapted from receiver operating characteristics-(ROC)-curve analyses, an assignment of urinary detected cellular components and renal allograft rejection, including T-cell mediated rejection (TCMR), was enabled (22). The conceptual extension of these findings to other T-cell-mediated renal pathologies, for example, ICI-related nephrotoxicity, seems attractive. Moreover, we previously reported intrarenal PD-L1-positivity as a potential indicator of ongoing kidney damage, showing that renal irAEs correlate with detected PD-L1$^+$ kidney cells in the urine (20, 21). PD-L1 upregulation was also observed in other renal pathologies implying PD-L1 functions as a response signal to injuries within the kidneys (20).

Therefore, it is tempting to speculate that the non-invasive urinary detection of PD-L1$^+$ kidney cells may enable identifying patients at risk for developing nephrotoxicity related to ICI therapy by urine monitoring.

In summary, in light of the increased use of ICI treatments, we expect a sizable number of renal complications. Therefore, cost-efficient and easily performable diagnostic tools for non-invasive biomonitoring have become more critical to improving renal and overall survival in cancer patients receiving immunotherapy.

Ethics statement

All enrolled patients will provide written consent. The ethics committee of the University Medical Center Göttingen, Germany, approved the study protocol (Protocol Number 1/10/21). The trial is registered at https://www.drks.de (DRKS-ID: DRKS00030999).

Author contributions

BT conceived the study and edited the manuscript. EB established protocols and wrote the first draft. PK reviewed and edited the draft and is involved in patient care. AS, K-MT, and TO are directly involved in the treatment of cancer patients. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Immunotherapy-related adverse events in real-world patients with advanced non-small cell lung cancer on chemoimmunotherapy: a Spinnaker study sub-analysis

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Background: The Spinnaker study evaluated survival outcomes and prognostic factors in patients with advanced non-small-cell lung cancer receiving first-line chemoimmunotherapy in the real world. This sub-analysis assessed the immunotherapy-related adverse effects (irAEs) seen in this cohort, their impact on overall survival (OS) and progression-free survival (PFS), and related clinical factors.

Methods: The Spinnaker study was a retrospective multicentre observational cohort study of patients treated with first-line pembrolizumab plus platinum-based chemotherapy in six United Kingdom and one Swiss oncology centres. Data were collected on patient characteristics, survival outcomes, frequency and severity of irAEs, and peripheral immune-inflammatory blood markers, including the neutrophil-to-lymphocyte ratio (NLR) and systemic immune-inflammation index (SII).

Results: A total of 308 patients were included; 132 (43%) experienced any grade irAE, 100 (32%) Grade 1–2, and 49 (16%) Grade 3–4 irAEs. The median OS in patients with any grade irAES was significantly longer (17.5 months [95% CI,

13.4–21.6 months]) than those without (10.1 months [95% CI, 8.3–12.0 months]) (p<0.001), either if Grade 1–2 (p=0.003) or Grade 3–4 irAEs (p=0.042). The median PFS in patients with any grade irAEs was significantly longer (10.1 months [95% CI, 9.0–11.2 months]) than those without (6.1 months [95% CI, 5.2–7.1 months]) (p<0.001), either if Grade 1–2 (p=0.011) or Grade 3–4 irAEs (p=0.036). A higher rate of irAEs of any grade and specifically Grade 1–2 irAEs correlated with NLR <4 (p=0.013 and p=0.018), SII <1,440 (p=0.029 ad p=0.039), response to treatment (p=0.001 and p=0.034), a higher rate of treatment discontinuation (p<0.00001 and p=0.041), and the NHS-Lung prognostic classes (p=0.002 and p=0.008).

Conclusions: These results confirm survival outcome benefits in patients with irAEs and suggest a higher likelihood of Grade 1–2 irAEs in patients with lower NLR or SII values or according to the NHS-Lung score.

KEYWORDS

lung cancer, immunotherapy, immune-related adverse effects, neutrophil-to-lymphocyte ratio (NLR), systemic immune-inflammation index (SII), overall survival, non-small cell lung cancer, progression free survival

Background

Lung cancer is the leading cause of cancer-related mortality worldwide, with most cases being non-small-cell lung cancer (NSCLC) (1, 2). The pharmacological management of patients with NSCLC has had major advancements as a result of the immunotherapy options now available (3–6). One such option is pembrolizumab, a programmed death-1 (PD-1) inhibitor. Its use alongside chemotherapy in patients with advanced NSCLC regardless of programmed death-ligand 1 (PD-L1) status has demonstrated improved survival outcomes and is now the standard of care (7).

With these immunotherapeutic options come a multitude of immunotherapy-related adverse effects (irAEs) affecting various bodily systems (3–6). However, previous analyses have reported that patients with irAEs tended to have better survival outcomes than patients without irAEs, but these were observed in trial cohorts (8). A retrospective study of patients with advanced NSCLC on immunotherapy alone also concluded that improved survival outcomes were seen among patients with irAEs. Another study of patients either on chemoimmunotherapy or immunotherapy alone at a German centre found that patients with irAEs survived longer though (9). However, data within a real-world cohort of patients who are solely on combined chemoimmunotherapy for their advanced NSCLC has yet to be presented.

The retrospective Spinnaker study assessed the efficacy of chemoimmunotherapy in patients with advanced NSCLC and subsequently established the NHS-Lung score as a tool to inform prognostic information in these patients (10). This score consisted of the following factors: a high number of metastatic sites, squamous histology of the tumour, and a high systemic immune-inflammatory index (SII). The present analysis following on from the Spinnaker study aims to assess the irAEs seen in this real-world patient cohort, the frequency and severity of these irAEs, and their impact on survival outcomes, and identify related clinical factors.

Materials and methods

The Spinnaker study was a retrospective multicentre cohort study, which included patients with histologically confirmed advanced NSCLC, no actionable genetic alterations, and any PD-L1 tumour proportion score (TPS). These patients were of Eastern Cooperative Oncology Group Performance Status (ECOG PS) ≤ 1. They received first-line chemotherapy alongside pembrolizumab at one of seven different centres (six in the United Kingdom and one in Switzerland) between March 2018 and April 2021 (10).

Data were collected on patient characteristics, tumour characteristics, survival outcomes, disease response, frequency and severity of irAEs, treatment discontinuation rates, and peripheral immune-inflammatory blood markers such as the neutrophil-to-lymphocyte ratio (NLR) and SII. NLR was derived from the ratio of the number of neutrophils to the number of lymphocytes measured from a blood count check of a peripheral blood test taken within 14 days of the treatment start date. A high NLR was defined as ≥4 as

Abbreviations: 95% CI, 95% confidence interval; CTC-AE, Common Terminology Criteria for Adverse Events; ECOG, Eastern Cooperative Oncology Group; irAEs, immunotherapy-related adverse effects; NHS-Lung, N = number of metastatic sites (cut-off ≥3), H = histology (i.e., squamous), S = SII (≥1440); NLR, neutrophil-to-lymphocytes ratio; NSCLC, non-small-cell lung cancer; OS, overall survival; PFS, progression-free survival; PS, performance status; SII, systemic immune-inflammation index; TPS, tumour proportion score.

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previously reported (11). The SII was calculated from the product of the NLR and the platelet count, with the cut-off threshold being ≥1,440 (12). The definition of irAEs was based on the causality established by the responsible physician in each participating centre between the AE and immunotherapy. The severity of the irAEs was agreed on by clinical judgement and graded referring to the common toxicity criteria (CTC)-AE version 5. In the subgroup analysis, patients who developed an irAE that started as Grade 1–2 before progressing to Grade 3–4 were counted as a single case of irAE of any grade.

The primary endpoint of this analysis was to describe the frequency, type, and severity of irAEs observed in these patients and how these affected their survival outcomes (i.e., overall survival (OS) and progression-free survival (PFS)). Secondary endpoints included assessing for possible clinical factors influencing the likelihood of irAEs.

Clinical data were analysed by descriptive statistics using percentages for binary variables and medians for continuous variables, with their respective dispersion values reported. The chi-square test was used when comparing binary variables, and a significance value of $p<0.05$ was defined. The OS was calculated from the treatment start date until death or the date of the last follow-up, and the PFS was calculated from the treatment start date to disease progression or death from any cause. Patients who had not had any events at the time of the analysis were censored. OS and PFS were estimated using the Kaplan–Meier method and reported as medians with 95% confidence limits (95% CI) and compared using a two-sided log-rank test with an acceptable significance value of $p<0.05$ (13). A Spearman correlation test was performed between the irAE subgroups (i.e., any grade, Grade 1–2, and Grade 3–4) and various patients and tumour and blood marker prognostic factors. We performed an exploratory Cox regression analysis according to the irAEs. As more than one organ toxicity may occur in the same patient, we first assessed the role of single versus multiple organ irAEs, then according to the type of single organ irAEs occurring in at least 10 patients. The statistical analysis was carried out by SigmaPlot software version 12.5 (Systat Software, San Jose, CA).

This study was registered and approved as an audit by the multiple participating sites, with the coordinating centre being Portsmouth Hospital University NHS Trust (United Kingdom). Clinical data were anonymised before sharing with the coordinating centre for analysis. The audit procedures were compliant with the Data Protection Act 2018, the precepts of Good Clinical Practice guidelines with regard to the collection, storage, processing, and disclosure of personal information, and the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations.

Results

Frequency, type, and severity of irAEs

The Spinnaker study included 308 patients from seven different centres (10). The characteristics of this patient cohort are described in Table 1. The median follow-up duration was 18 months (15.0–20.1 months). There were 132 cases of irAEs of any grade (43% of

TABLE 1 Patient characteristics and outcomes.

Characteristic	No. (%) [range]
Age	
Median	65 [37–84]
≥70 years	98 (32)
Gender	
Male	171 (56)
Female	137 (44)
Smoking history	
Never	25 (8)
Former	192 (62)
Current	91 (30)
Histology	
Squamous	51 (17)
Adenocarcinoma	246 (80)
Other[a]	11 (3)
ECOG PS	
0	127 (41)
1	181 (59)
Stage	
IIIB/IVA	24 (8)
IVA	113 (37)
IVB	171 (56)
BMI[b]	
Median	24.8 [15.0–43.9]
Underweight/normal	16 (5)/146 (47)
Overweight/obese	100 (32)/46 (15)
Number of metastatic sites	
≥ 3	103 (33)
Brain metastases	31 (10)
Liver metastases	37 (12)
PD-L1 IHC Ab[c]	
22C3/SP263	145 (49)/151 (51)
Negative	165 (56)
Positive	111 (37)
High	20 (7)
N/A	12 (4)
Oncogene (EGFR/ALK/ROS1)	3 (1)
Pre-treatment steroids	33 (11)
NLR ≥4	164 (53)
SII ≥ 1,444	154 (50)

(Continued)

TABLE 1 Continued

Characteristic	No. (%) [range]
Type of chemotherapy	
Cisplatin-Pemetrexed	24 (8)
Carboplatin-Pemetrexed	240 (78)
Carboplatin-Paclitaxel	44 (14)
Best response[d]	
CR	2 (1)
PR	197 (67)
SD	52 (18)
PD	45 (15)
N/A	12 (2)
GCSF given	59 (19)
irAE	
Any grade	132 (43)
G1–G2	100 (32)
G3–G4	49 (16)
Treatment discontinuation[e]	72 (23)
Median follow up (months) [95% CI]	18.0 [15.9–20.1]
Median OS (months) [95% CI]	12.7 [10.2–15.2]
Median PFS (months) [95% CI]	8.0 [7.1–8.8]

Ab, antibody; BMI, body mass index; CI, confidence interval; CR, complete response; ECOG PS, Eastern Cooperative Oncology Group Performance Status; GCSF, granulocyte colony-stimulating factor; IHC, immunohistochemistry; mo., months; NA, not assessable; NLR, neutrophil-to-lymphocyte ratio; No. Number; OS, overall survival; PD-L1, programmed cell death-ligand-1; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease; SII, systemic immune-inflammatory index; TPS, tumour proportion score; yr, year.

[a]Including poorly differentiated (No. 6), undifferentiated (No. 2), sarcomatoid (No. 1), adenosquamous (No. 1), and pleomorphic (No. 1) histology.

[b]BMI was calculated using the formula of weight/height^2 (kilograms/square metres) and categorized according to the World Health Organization (WHO) categories: underweight (BMI<18.5), normal weight (18.5≤BMI ≤ 24.9), overweight (25≤BMI ≤ 29.9), obese (BMI≥30).

[c]Negative, TPS >1%; positive, TPS 1-49%; high, TPS ≥ 50%.

[d]By RECIST version 1.1 criteria.

[e]Due to toxicity.

patients). One hundred patients (32%) developed Grade 1–2 irAEs, and 49 patients (16%) had Grade 3–4 irAEs. Table 2 describes the range of irAEs seen and their frequency. The three most common bodily systems affected by irAEs of any grade were the skin (12%), bowel (7%), and thyroid (7%). This distribution was similar for Grade 1–2 irAEs. The most frequently seen Grade 3–4 irAEs were colitis and pneumonitis (5% each) and hepatitis and skin toxicity (2% each). A total of 72 patients (23%) discontinued treatment due to toxicity.

Association of irAEs with survival outcomes

The median OS was significantly longer in all three subgroups of patients with any grade, Grade 1–2, and Grade 3–4 irAEs than in patients without irAEs (Table 3, Figure 1). Patients with irAEs of any grade had a median OS of 17.5 months (95% CI, 13.4–21.6 months), while patients without these irAEs had a median OS of 10.1 months (95% CI, 8.3–12.0 months) (p<0.001). Patients experiencing Grade 1–2 irAEs had a significantly longer median OS of 16.6 months (95% CI, 12.6–20.6 months) compared to those without who had a median OS of 11.8 months (95% CI, 10.1–13.6 months) (p=0.003). Patients experiencing Grade 3–4 irAEs also had a significantly longer median OS of 24.0 months (95% CI, 9.0–39.1 months) compared to those without who had a median OS of 12.1 months (95% CI, 9.8–14.5 months) (p=0.042).

The median PFS was significantly longer in all three subgroups of patients with any grade, Grade 1–2, and Grade 3–4 irAEs than in patients without irAEs (Table 4, Figure 2). Patients with irAEs of any grade had a median PFS of 10.1 months (95% CI, 9.0–11.2 months), while patients without these irAEs had a median PFS of 6.1 months (95% CI, 5.2–7.1 months) (p<0.001). Patients experiencing Grade 1–2 irAEs had a significantly longer median PFS of 9.6 months (95% CI, 8.1–11.1 months) compared to those without who had a median PFS of 7.0 months (95% CI, 5.9–8.1 months) (p=0.011). Patients experiencing Grade 3–4 irAEs also had a significantly longer median PFS of 10.5 months (95% CI, 7.2–13.7 months) compared to those without who had a median PFS of 7.5 months (95% CI, 6.4–8.5 months) (p=0.036).

Correlation of irAE with clinical prognostic factors

In the groups of patients with irAEs of any grade and those specifically with irAEs of Grade 1–2, a higher frequency of irAEs occurred in patients with NLR <4 (p=0.013 and p=0.018, respectively), SII <1,440 (p=0.029 and p=0.039, respectively), lower NHS-Lung score (p=0.002 and p=0.008, respectively), better disease response (p=0.001 and p=0.034, respectively) and if their treatment had been discontinued (p<0.00001 and p=0.041, respectively). In patients with Grade 3–4 irAEs, however, a higher frequency of irAEs was observed only in patients with better disease response (p=0.039) and if treatment had been discontinued (p=0.0001). There were no associations detected between the occurrence of irAEs and gender, pre-treatment PS, or PDL1 TPS.

Association of irAEs type with survival outcomes

Both single and multiple organ irAEs were significantly associated with longer OS (p<0.001 and p=0.032, respectively), whereas only single-organ irAEs were significantly associated with longer PFS (p=0.002 and p=0.056). Within the limits of a non-landmark analysis, among single-organ irAEs, thyroid irAEs were significantly associated with both longer OS and PFS (p=0.009 and p=0.032), whereas skin irAEs were associated with longer OS (p=0.032) but not PFS (p=0.066) (Supplementary Table S3, S4).

TABLE 2 Immunotherapy-related adverse effects.

irAE, No. (%)	Any grade No (%)	G1–G2 No (%)	G3–G4 No (%)
Any irAE	132 (43)	100 (32)	49 (16)
Skin	44 (14)	37 (12)	7 (2)
Colitis[a]	37 (12)	21 (7)	16 (5)
Thyroid	22 (7)	21 (7)	1 (0)
Pneumonitis	25 (8)	11 (4)	14 (5)
Liver[b]	16 (5)	9 (3)	7 (2)
Nephritis[b]	9 (3)	7 (2)	2 (1)
Hypophysitis	7 (2)	5 (2)	2 (1)
Arthritis	4 (1)	3 (1)	1 (0)
Adrenal	2 (1)	1 (0)	1 (0)
Myasthenia	1 (0)	0 (0)	1 (0)

[a]Only one toxic death in the whole series attributed to immunotherapy-related colitis.
[b]Includes both laboratory abnormalities and diagnosis.

Discussion

The results of this analysis have shown that patients with irAEs of any grade had better survival outcomes regardless of the grade of the irAE. Potential predictors for the development of Grade 1–2 irAEs have also been identified including the NLR, the SII, the NHS-Lung score, disease response, and treatment discontinuation. Grade 3–4 irAEs were predicted only by disease response and treatment discontinuation. A possible explanation for the lack of correlation observed between Grade 3–4 irAEs and the other factors listed above could be due to the relatively low incidence of Grade 3–4 irAEs. The main limitations of this analysis include its retrospective nature and the lack of information on the timing of the irAEs observed. Nevertheless, the Spinnaker study was a multicentre project with a real-life cohort of patients lending itself to the generalisability of the results of this present analysis.

A previous pooled analysis of the IMpower130, IMpower132, and IMpower150 trials mirrored the findings from this analysis. It noted that patients with advanced NSCLC on a combination of chemotherapy, atezolizumab, and/or bevacizumab who experienced irAEs had longer OS compared to those without (8). This was also reflected in the outcomes of retrospective studies of patients with NSCLC who had received immunotherapy (9, 14). A previous work has shown that concurrent GCSF prophylaxis use in a proportion of patients in this cohort had no confounding impact on survival outcomes (15).

A systematic review of 51 studies assessing the use of immunotherapy in various solid malignancies, including lung cancers, detected a positive association between the development of irAEs and survival outcomes (16). Other works have echoed these findings among a variety of tumours being treated with immunotherapy (17–20). However, this present analysis highlights these associations between the presence of irAEs and improved survival outcomes among patients being treated with combined chemoimmunotherapy.

The results reported could be explained by a higher efficacy of immunotherapy in patients experiencing irAEs, thus conferring improved survival outcomes but potentially more irAEs. Therefore, the presence of irAEs could serve as a useful

TABLE 3 Overall survival according to grade of immunotherapy-related adverse effects.

irAE		No.	Median [95% confidence interval]	p-value
Any irAE	No	176	10.1 [8.3–12.0]	p < 0.001
	Yes	132	17.5 [13.4–21.6]	
G1–2 irAE	No	205	11.8 [10.1–13.6]	p = 0.003
	Yes	100	16.6 [12.6–20.6]	
G3–4 irAE	No	256	12.1 [9.8–14.5]	p = 0.042
	Yes	49	24.0 [9.0–39.1]	

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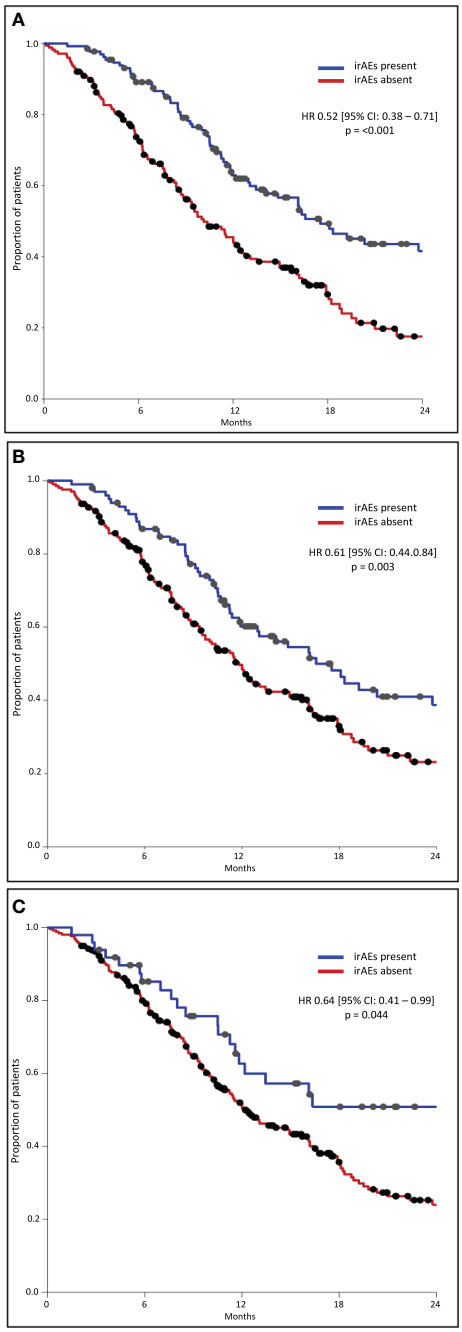

FIGURE 1
Overall survival – any grade **(A)**, G1-2 **(B)**, G3-4 **(C)**.

indicator for treatment response and survival. The potential for an immortal bias is also recognized, with possibly more irAEs being detected in patients surviving longer. A landmark analysis or a time-dependent Cox analysis could not be performed as the time of the immunotherapy-related adverse event was not recorded. Notably, in the present analysis, patients with irAEs had a higher rate of treatment discontinuation potentially indicating a low impact of the length of immunotherapy treatment on the improved survival outcomes.

There have been a number of reports suggesting potential predictive markers to identify patients who are more likely to develop irAEs. A prospective cohort study of patients with a solid or haematological malignancy in a French cancer centre found severe irAEs in those who had a PS ≥2 (21). All patients in the Spinnaker study were of PS 0–1, and therefore, a similar comparison to PS ≥2 cannot be made. However, Ruste et al. also reported that patients with a high NLR had severe irAEs. This contradicts this present analysis' finding of an association between an NLR of <4 and the development of irAEs. Our findings are supported by other works that found a higher frequency of irAEs in patients with a low NLR (22–25) and the fact that the presence of irAEs is consistently associated with better survival outcomes and a low NLR is a prognostic indicator and predictive marker of response to immunotherapy in advanced NSCLC (11, 26, 27). The NLR and/or SII have already been incorporated in prognostic scoring tools for these patients such as the NHS-Lung score, Lung Immune Prognostic Index (LIPI), and Lung Immune Prognostic score (LIPS) (10, 28–31). In addition to this, other works have highlighted the use of interleukin-6 (IL-6) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) levels and tumour burden to predict the occurrence of irAEs (32–34).

Conclusions

The results of this retrospective analysis have shown that patients with irAEs had better survival outcomes. It has identified potential predictors of patients developing irAEs, including the NLR score, the SII score, the NHS-Lung score, disease response, and treatment discontinuation. The NHS-Lung score is an easy-to-use tool that can help predict not only prognoses in patients with advanced NSCLC on

TABLE 4 Progression-free survival according to grade of immunotherapy-related adverse effects.

irAE		No.	Median [95% confidence interval]	p-value
Any irAE	No	176	6.1 [5.2–7.1]	p < 0.001
	Yes	132	10.1 [9.0–11.2]	
G1–2 irAE	No	205	7.0 [5.9–8.1]	p = 0.011
	Yes	100	9.6 [8.1–11.1]	
G3–4 irAE	No	256	7.5 [6.4–8.5]	p = 0.036
	Yes	49	10.5 [7.2–13.7]	

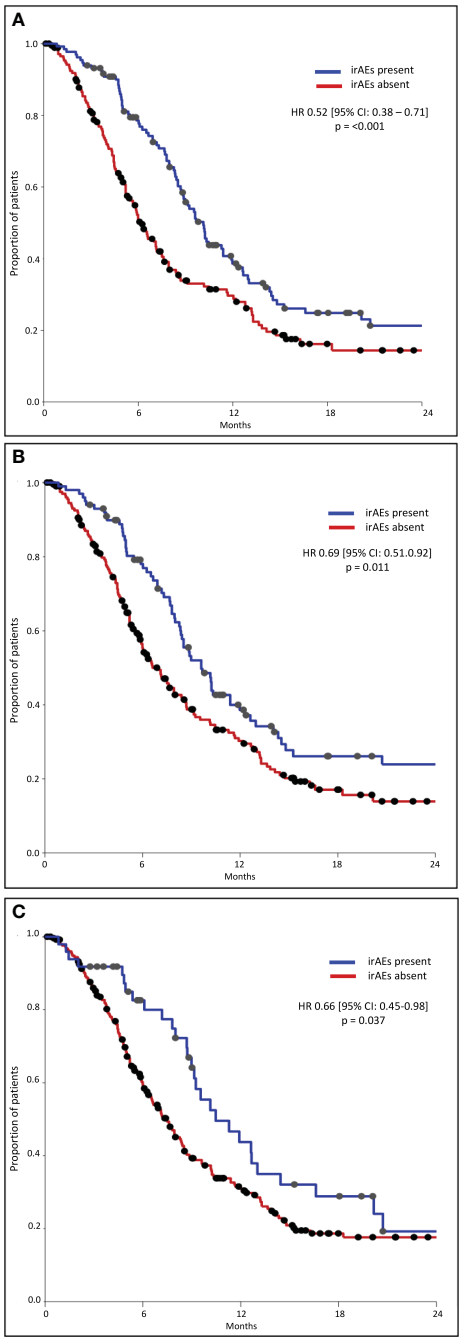

FIGURE 2
Progression-free survival – any grade **(A)**, G1-2 **(B)**, G3-4 **(C)**.

chemoimmunotherapy but also the likelihood of irAEs. The use of these scores may lead to a more proactive approach to identifying patients at risk of irAEs and therefore their prompt management, avoiding these irAEs progressing in severity.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

Conceptualisation: GB, AA, FG Methodology: GB, AS Software: GB, AS Validation; AS Formal analysis: GB, AS Investigation: JC, AC, CE, DP, CO, CC, SCh, SM. Resources: LM, SCa Data curation: PH, RB, CC, AC, AA, HM, GB Original draft: SA, GB Supervision: GB, CE, AA Project administration: LM, SCa All authors contributed to manuscript revision and approved the submitted version. FG and GB contributed equally as co-last authors.

Conflict of interest

GB received grant consultancies from Astrazeneca and Astellas Pharma. AC received speaker fees and grant consultancies by Astrazeneca, MSD, IQVIA, OncoC4, and EISAI. AA received consulting fees from BMS, Astrazeneca, Boehringer-Ingelheim, Roche, MSD, Pfizer, Eli Lilly, and Astellas, and speakers fees from Eli Lilly and Astrazeneca. DP received lecture fees from ViiV Healthcare, Bayer Healthcare, BMS, Roche, EISAI, and Falk Foundation; travel expenses from BMS and Bayer Healthcare; consulting fees for Mina Therapeutics, EISAI, Roche, Avamune, Exact Sciences, Mursla, DaVolterra, and Astra Zeneca; and research funding to institution from MSD and BMS. GB received travel expenses from Novartis. CO reports personal fees from BMS.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1163768/full#supplementary-material

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Case Report: Imaging immune checkpoint inhibitor-induced yin-yang effects in the brain

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Background: Treatment with immune checkpoint inhibitors (ICI) can induce durable responses in cancer patients, but it is commonly associated with serious immune-related side effects. Both effects are suggested to be mediated by CD8+ T-cell infiltration. Whole body CD8+ T-cell distribution can be visualized by PET imaging of a 89Zr-labeled anti-humanCD8a minibody, currently investigated in a phase 2b trial.

Main body: An adult patient diagnosed with metastatic melanoma developed ICI-related hypophysitis after two courses of combined immunotherapy (ipilimumab (3 mg/kg) and nivolumab (1 mg/kg) at 3 weeks interval). On a [^{89}Zr]Zr-crefmirlimab berdoxam PET/CT scan, made 8 days before clinical symptoms occurred, increased CD8+ T-cell infiltration in the pituitary gland was detected. Simultaneously, tracer uptake in a cerebral metastasis was increased, indicating ICI-induced tumor infiltration by CD8+ T-cells.

Conclusions: The observations in this case report underscore the role of CD8+ T-cell in non-tumor tissues in ICI-related toxicity. In addition, it illustrates a potential role for molecular imaging by PET/CT for investigation and monitoring of ICI-induced effects.

KEYWORDS

PET/CT imaging, immune checkpoint inhibitors, CD8+ T-cells, toxicity, melanoma

Highlights

Non-invasive CD8+ T-cell tracking in patients visualizes CD8+ T-cell infiltration in tumor, as well as in healthy organs. This novel technology is under investigation to assess its predictive potential, but may also allow early monitoring of immune checkpoint inhibitor-related toxicity.

Introduction

Immune checkpoint inhibition has revolutionized the treatment of metastatic melanoma patients (1). Combined targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed death ligand-1 (PD-L1) maximizes cytotoxic (CD8+) T-cell activation, resulting in higher objective response rates and an improved median overall survival as compared to checkpoint inhibitor monotherapy (2). Increased T-cell activation is also associated with immune-related adverse events, and severe toxicities occur frequently under combination treatment (1, 3). Immune-related adverse events can be life-threatening and may warrant early and aggressive immunosuppressive treatment, but tools for early detection are lacking (4). In this report, we present a case with immune-related hypophysitis, which was detected with PET imaging of a ^{89}Zr-labeled anti-hCD8α minibody, with the product name [^{89}Zr]Zr-crefmirlimab berdoxam (5), to non-invasively track CD8+ T-cells, 8 days before clinical presentation.

Case presentation

An adult male patient 73 years of age was diagnosed with metastatic melanoma localized in the brain, gallbladder, ampulla of Vater, and left maxillary sinus, as determined by contrast-enhanced CT scan of chest and abdomen, and dynamic contrast-enhanced MRI of the brain. The patient had one solitary brain metastasis of which the largest diameter was 32 mm. Lactate dehydrogenase was normal and ECOG performance score was 1. A *BRAF*^{V600E} mutation was present but patient agreed to the preferred first-line of treatment with combined immunotherapy consisting of ipilimumab (3 mg/kg) and nivolumab (1 mg/kg) at 3 weeks interval. Patient consented to participate in study NCT05013099, a phase 2b study investigating PET/CT scans with [^{89}Zr]Zr-crefmirlimab berdoxam, a radiolabeled anti-hCD8α minibody. PET/CT scans were made at baseline and after two courses of combination treatment, to evaluate treatment induced changes in CD8+ T-cell distribution *in vivo*. A week after the 3rd course of immunotherapy patient presented with general malaise, weakness, headache, decreased appetite and feeling cold. Laboratory evaluation showed mild hyponatremia (sodium 127 mmol/l; reference, 135-145), an elevated C-reactive protein (53 mg/l; reference, <10), and decreased hormone levels of multiple endocrine axes (cortisol 0.13 µmol/l at 9:20 a.m.; reference, 0.19-0.55: adrenocorticotropic hormone 1.4 pmol/l; reference 1.6-13.9: TSH 0.26 mE/l; reference, 0.27-4.20: FT4 5.2 pmol/l; reference, 10.0-23.0: FSH 3.5 E/l; reference 1.5-12: LH 0.79 E/l; reference, 1.7-8.6: testosterone <0.42 nmol/l; reference, 10.5-37: prolactin 17 mE/l; reference, 86-320). Insulin-like growth factor, lactate dehydrogenase and potassium levels were normal. Physical examination revealed no abnormalities except for a decreased blood pressure of 124/65 mmHg (previous blood pressures 160-180/80 mmHg) with a pulse of 91/min. The clinical diagnoses of immune checkpoint inhibitor-related hypophysitis affecting multiple endocrine axes was made. Hormonal replacement therapy with hydrocortisone (20 mg/day) and levothyroxine (50 µg/day, later increased to 75 µg/day) was initiated after which the symptoms improved rapidly. The fourth course of combined immunotherapy was not given due to an adrenal crisis despite start of hormonal treatment. Patient was admitted with symptoms of headache and fever and treated with high dose hydrocortisone after which he recovered quickly. An intercurrent problem triggering the adrenal crisis was not diagnosed and the patient was discharged from the hospital after 4 days. Planned tumor evaluation 13 weeks after start of ipilimumab plus nivolumab showed stable disease per RECIST 1.1, including the cerebral metastasis, and no new lesions. Patient continued nivolumab monotherapy as planned.

Retrospective analyses of the [^{89}Zr]Zr-crefmirlimab berdoxam PET scan showed an increase in tracer uptake in the pituitary gland after two cycles of combination treatment, from maximum standardized uptake value (SUV_{max}) 1.3 to SUV_{max} 3.7, suggesting treatment-induced increased infiltration of CD8+ T-cells (Figure 1). In parallel, the tracer uptake in the cerebral metastasis increased 2.8-fold, from (SUV_{max}) 1.6 at baseline to SUV_{max} 4.5, suggesting increased CD8+ T-cell tumor infiltration. Imaging preceded the clinical presentation of ICI-induced hypophysitis by 8 days. Moreover, one day prior to the scan the patient was seen at our out-patient clinic and showed no symptoms of hypophysitis. On the day of the scan the patient had a normal potassium value and only mildly lowered thyroid hormone values (TSH 0.50 mE/l; FT4 8.7 pmol/l). No increase in tracer uptake was observed in downstream endocrine organs, ie. thyroid gland or adrenal glands (Table 1; Supplementary Table S1).

Discussion

ICI-related hypophysitis is an infrequent but potentially serious adverse event that occurs more often in patients treated with combination immunotherapy compared to monotherapy (6, 7). Like other endocrinopathies, pituitary dysfunction is irreversible and patients require lifelong hormone replacement therapy. High-dose corticosteroids are rarely indicated and ICI treatment can be continued in most patients (8).

[^{89}Zr]Zr-crefmirlimab berdoxam is a humanized minibody that targets the α-subunit of human CD8 (5) for the purpose of *in vivo* tracking of CD8+ T-cells, as main effector cells of anti-cancer immunity. Its capacity to predict response to checkpoint inhibition and detect toxicity is currently being investigated in a multi-center phase 2b study (NCT05013099) in which this patient participates. Similarly, CD8+ cytotoxic T-cells are suggested as mediators of ICI-related toxicities, however, reports on direct correlation are limited (9) as non-invasive tools to track CD8+ T-cells in patients were lacking. Conventional medical imaging as magnetic resonance imaging may reveal tissue changes commonly related to inflammation, e.g. swelling, edema and/or fibrosis (10) at clinical presentation, however, the role of anatomical imaging modalities in early detection is not investigated. As ICI-related toxicity is likely accompanied by other features of inflammation such as increased perfusion, this may also contribute to increased signal on PET. Although the increasing ratio of tracer uptake in the pituitary gland over bloodpool activity suggests

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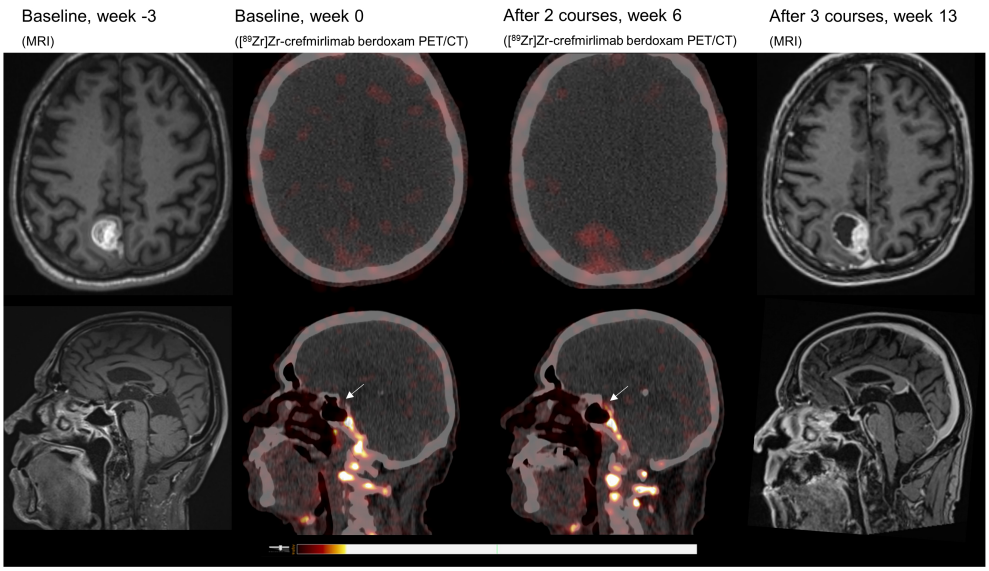

FIGURE 1
MRI and [^{89}Zr]Zr-crefmirlimab berdoxam PET/CT scans of cerebral lesion and pituitary gland.

accumulation of the tracer in the interstitial space, further research should confirm that the PET signal is due to CD8+ T-cell infiltration. In addition, the method of quantification of PET signal and definition of organ-specific threshold values associated with toxicity is currently under investigation.

This case demonstrates that CD8+ PET imaging has the potential to evaluate CD8+ trafficking to tissues at risk for ICI-related toxicity at early timepoints during treatment, and warrants further exploration of this modality in relation to management of severe ICI-related toxicity.

TABLE 1 Lesion size and tracer uptake measurements on baseline and follow up imaging.

	Baseline	Baseline	After 2 courses of treatment	After 3 courses of treatment
	Largest diameter, CT/MRI (mm)	Tracer uptake, PET (SUV_{max})	Tracer uptake, PET (SUV_{max})	Largest diameter, CT/MRI (mm)
Tumor lesion				
Cerebral	18.9	1.6	4.5	18.5
Gallbladder	43	2.9	3.6	39.8
Left maxillary sinus	17.1	2.2	1.5	15.1
Ampulla of Vater	non-measurable			
Organ system				
Pituitary gland		1.3	3.7	
Thyroid gland		3.1	1.5	
Adrenal gland		4.0	2.9	
Liver		13.6	15.5	
Bloodpool		1.2	1.6	
Spleen		61.1	60.1	
Bone marrow		14.6	17.1	

Conclusion

Non-invasive CD8+ T-cell tracking in patients treated with immune checkpoint inhibitors revealed not only increased tracer accumulation in the brain metastasis, but also in ICI-related hypophysitis on a PET/CT scan made 8 days prior to onset of symptoms. These observations suggest that PET-based CD8+ T-cell trafficking in patients is a potential tool for early monitoring of ICI-related response and toxicity.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by METC Oost-Nederland. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2023.1199282/full#supplementary-material

SUPPLEMENTARY TABLE 1
Tracer uptake in reference tissues on baseline and follow up imaging.

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Pancreatic adverse events of immune checkpoint inhibitors therapy for solid cancer patients: a systematic review and meta-analysis

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Objective: This review aims to determine the incidence and risk of pancreatic adverse events (AEs) associated with immune checkpoint inhibitors (ICIs) therapy for solid tumors.

Methods: We conducted a comprehensive systematic literature search in PubMed, Embase, and Cochrane Library up to March 15, 2023, to identify all randomized controlled trials comparing ICIs with standard treatment in solid tumors. We included studies that reported immune-related pancreatitis or elevation of serum amylase or lipase levels. Following protocol registration in PROSPERO, we conducted a systematic review and meta-analysis.

Results: 59 unique randomized controlled trials with at least one ICI-containing arm (41 757 patients) were retrieved. The incidences for all-grade pancreatitis, amylase elevation and lipase elevation were 0.93% (95% CI 0.77-1.13), 2.57% (95% CI 1.83-3.60) and 2.78% (95% CI 1.83-4.19), respectively. The incidences for grade ≥3 pancreatitis, amylase elevation and lipase elevation were 0.68% (95% CI 0.54-0.85), 1.17% (95% CI 0.83-1.64) and 1.71% (95% CI 1.18-2.49), respectively. The use of ICIs was associated with an increased risk of all-grade pancreatic immune-related AEs (irAEs) including pancreatitis (OR=2.04, 95% CI 1.42-2.94, P =0.0001), amylase elevation (OR=1.91, 95% CI 1.47-2.49, P < 0.0001) and lipase elevation (OR=1.77, 95% CI 1.37-2.29, P < 0.0001). In addition to these, the *post-hoc* analysis found that PD-1 inhibitors had a significant higher risk of pancreatic AEs compared with PD-L1 inhibitors and the patients undergoing dual ICI therapy were at a significantly higher risk of pancreatic AEs than the patients receiving single ICI therapy.

Conclusion: Our study provides an overview of the incidence and risk of ICI-associated pancreatitis and pancreatic enzyme elevations in the treatment of solid tumors. Our findings may help raise awareness among clinicians of the potential for ICI-associated pancreatic AEs in clinical practice.

Systematic review registration: https://www.crd.york.ac.uk/PROSPERO, identifier 345350.

KEYWORDS

pancreatic adverse events, drug-related adverse events, immune checkpoint inhibitors, immunotherapy, meta - analysis

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Introduction

Immune checkpoint inhibitors (ICIs) including programmed cell death 1 (PD-1) inhibitors, programmed cell death 1 ligand 1 (PD-L1)inhibitors and cytotoxic T- lymphocyte-associated antigen 4 (CTLA-4) inhibitors have revolutionized cancer therapy and become the standard treatment for a number of malignancies in the past few years (1, 2). While ICIs activate the immune system against tumor cells, they can also lead to adverse events due to the imbalance of immunologic homeostasis in normal tissues (3). IrAEs can range from mild self-limiting symptoms to severe life-threatening events that can affect nearly all organ systems. These adverse events include but are not limited to, colitis, hepatitis, dermatitis, pneumonia, endocrine disorders, nephritis, myocarditis, and neuropathy (4). As the use of immunotherapy in cancer patients continues to rise, uncommon irAEs present a significant clinical challenge (5). Pancreatic AEs are rare but often overlooked, requiring clinician attention due to their adverse impact on the quality of life of cancer patients.

Despite early clinical studies confirming the immune-related toxicity of ICIs in the pancreas (6), several questions remain unanswered. Firstly, how to effectively recognize pancreatic irAEs, as they may present as asymptomatic elevations in amylase and/or lipase levels, as per the guidelines of the National Comprehensive Cancer Network (NCCN) (7). Furthermore, it is unclear whether the incidence of pancreatic AEs increases with the widespread use of ICIs and whether different types of combination therapy affect the risk of incidence. Therefore, our study aims to address these knowledge gaps and provide insights into predicting and managing pancreatic irAEs through a systematic review and meta-analysis.

Methods

Search strategy

This systematic review and meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (8). The statement was registered at the International Prospective Register of Systematic Reviews (number 345350). We conducted a comprehensive systematic literature search in PubMed, Embase, and Cochrane Library up to March 15, 2023, for all randomized controlled trials(RCTs)that compared ICIs with standard treatment in solid tumors. Based on PICOS (participants, interventions, comparisons, outcomes, and study design) guidelines (9), the keywords and Medical Subject Headings (MeSH) terms were used as follows: “neoplasms”; “immune checkpoint inhibitor”, “PD-1 inhibitors”, “PD-L1 inhibitors”,” CTLA4 inhibitors” “pembrolizumab”, “nivolumab”, “tislelizumab”, “sintilimab”, “camrelizumab”, “toripalimab”, “atezolizumab”, “avelumab”, “durvalumab”, “cemiplimab”, “tremelimumab”, “Ipilimumab” “drug-related side effects and adverse reactions”, “adverse reactions”, and “randomized controlled trials”.

Selection criteria

Studies eligible for inclusion met all the following criteria: (1) phase III RCTs including at least one ICI-containing arm (ICIs as monotherapy or in combination with another ICIs or standard treatment) in adult patients (age >_18 years) with solid cancer; (2) clinical trials reporting immune-related pancreatitis or elevation of serum amylase or lipase levels; and (3) studies published in English. The exclusion criteria were as follows: (1) studies published as abstracts, letters, or conference reports; (2) studies published repeatedly; (3)both treatment arms were immunotherapy.

Data extraction

Two investigators (ZZ and WZ) independently evaluated the titles, abstracts, and full texts to select the potentially eligible publications. The following data were obtained from the included study: basic information (first author, publication year, trial name, and Clinical Trial number), participants(disease diagnosis, treatment arms, and the number of included patients), and the number of patients with pancreatitis, amylase elevation, and lipase elevation for all-grade (G1–5) and for grade 3 or higher (G3–5). The severity of the AE was graded on a scale from 0 to 5, with grade 0 being no toxicity and grade 5 being death according to the Common Terminology Criteria for Adverse Events(CTCAE) (10). Additional data included ICI regimen, control arm regimen, previous lines of chemotherapy, blindness, and median/mean follow-up (months). The primary outcome of our meta-analysis was the summary risk of pancreatic AEs associated with ICI exposure (ICIs as monotherapy or in combination with other ICIs or standard treatment) vs. controls in RCTs. If disagreement occurred, it was resolved by discussion with the corresponding author. All included studies represented unique trials.

Statistical analysis

To conduct a meta-analysis of the incidence and profile of pancreatic AEs, a random effect model with logit transformation was applied. All models are fitted by restricted maximum likelihood estimation with a classic continuity correction of 0.5 for zero cells and the corresponding sample sizes. Multiple groups of a trial were combined separately. The outcome measure is the incidence with its 95% confidence interval (CI). Based on previous studies (11), we hypothesized that pancreatic AEs are not a frequent event (incidence < 10%), and we interpreted the odds ratio(OR) as a measure of risk (12, 13). Pooled ORs and 95% CIs were estimated with a random effects model using the Mantel–Haenszel method (14). If a study included more than one intervention arm, we separately compared each intervention arm with the control arm. In addition to that, we conducted subgroup analyses to examine studies by cancer type and combination type.

Post-hoc analyses were used to assess the pancreatic AEs differences between anti-PD-1 drugs and anti-PD-L1 drugs, as

well as, between dual- and single -ICI therapies. We matched the included RCTs with their tumor type and intervention type, or tumor type and design of control groups to form several mirror groups for the adjusted indirect comparison (15). An OR (95% CI) was derived from each mirror group and then pooled across all ICI groups using a random-effects model.

We used the inconsistency index I^2 statistic and $\chi 2$ test with its P-value to evaluate the heterogeneity between studies. According to the Cochrane Handbook for Systematic Reviews of Interventions, substantial heterogeneity between studies was defined by I^2 value > 50%, and significant heterogeneity was defined by $\chi 2$ test P-value <0.10 (16). Publication bias was assessed using Peter tests with funnel plots, which is a recommended method for dichotomous data with low heterogeneity (17, 18). The risk of bias of included studies were evaluated with the Cochrane risk of bias tool (19). All analyses were done using Review Manager 5.3 software (Cochrane Collaboration 2014, Nordic Cochrane Center, Copenhagen, Denmark) and R statistical software (version 4.1.3; with the metafor_v3.0–2 packages) (20). A two-sided P-value of <0.05 in Z-tests (for overall effect) or $\chi 2$ test (for overall subgroup comparison) in all analyses was considered statistically significant.

Results

Eligible studies and characteristics

We identified 25 874 records from PubMed, Embase, and Cochrane Library. Figure 1 and Supplementary Table 1 illustrate the details of the study screening and selection procedures. Finally, 59 eligible studies involving 41 757 patients for quantitative analysis were included. Details of the study characteristics are presented in Table 1. Among these 59 RCTs, one was a four-arm study and 9 RCTs were three-arm. The mean follow-up time for the entire population ranged from 7.3 to 41.2 months. According to the type of combination therapy, there were 30 arms of ICI monotherapy 32 arms of ICI plus chemotherapy or targeted therapy, and 8 arms of dual-ICI therapy. In our study, we incorporated multiple tumor types including non-small cell lung cancer (NSCLC, n =19) (21–39), small cell lung cancer(SCLC, n = 3) (40–42), melanoma (n = 6) (43–48), gastroesophageal junction cancer (GEJC, n = 6) (49, 51, 52, 54, 80, 81), urothelial carcinoma (UC, n = 4) (55–58), renal cell carcinoma (RCC, n=4) (59–62), breast cancer (BC,n=1) (63), head and neck squamous cell carcinoma (HNSCC, n=3) (64–66), prostate cancer (PC,n=1) (67), hepatocellular

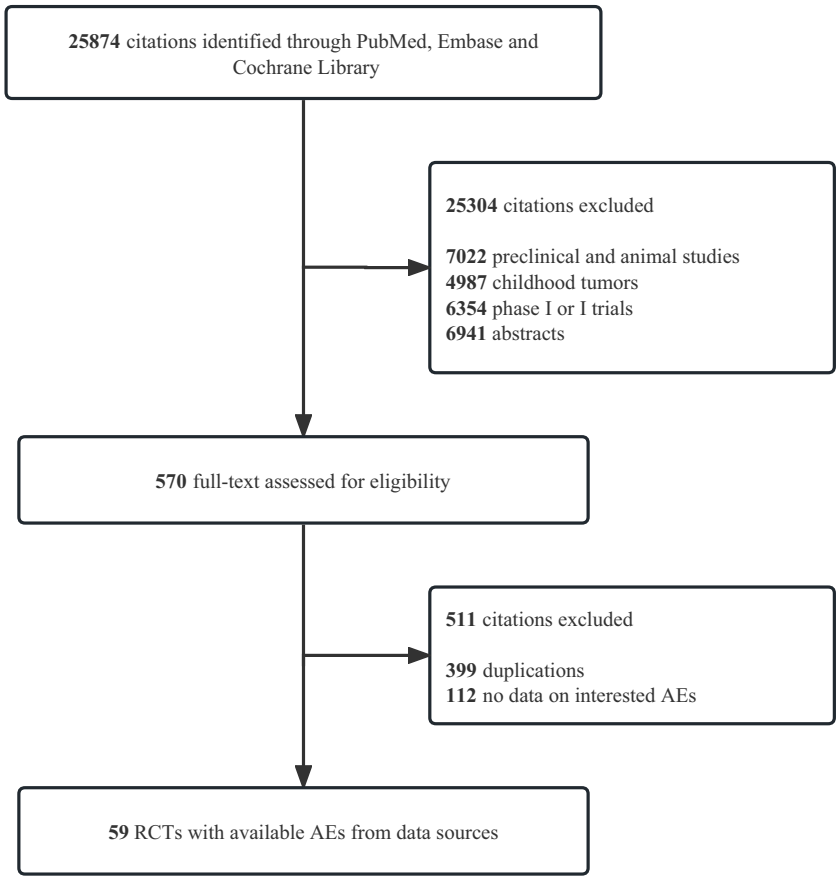

FIGURE 1
Study flow diagrams. PRISMA flow diagram of systematic review and meta-analysis in PubMed, Emabse, and Cochrane Library up to March 15, 2023. AEs, adverse events; RCTs, randomized controlled trials; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

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TABLE 1 Characteristics of the randomized clinical trials included in the meta-analysis.

Study (Year)	Trial name (Clinical Trials.gov Identifier)	Type of cancer	Treatment arm	Patient (no.)	Pancreatitis (G1-5)	(G3-5)	AMY (G1-5)	(G3-5)	Lipase (G1-5)	(G3-5)
D. Planchard (2020) (21)	ARCTIC (NCT02352948)	NSCLC	Durvalumab	117	0	0	2	1	0	0
			Durvalumab+ Tremelimumab	173	2	2	4	0	0	0
			Tremelimumab	60	0	0	0	0	1	1
			Chemotherapy	110	0	0	0	0	0	0
Martin Reck (2019) (22)	KEYNOTE-024 (NCT02142738)	NSCLC	Pembrolizumab	154	1	1				
			Chemotherapy	150	0	0				
Martin Reck (2020) (23)	IMpower150 (NCT02366143)	NSCLC	Atezolizumab+ Bevacizumab + Chemotherapy	393	5					
			Atezolizumab+ Chemotherapy	400	2					
			Chemotherapy	394	0					
Yi-Long Wu (2019) (24)	CheckMate 078 (NCT02613507)	NSCLC	Nivolumab	337			1	1	1	1
			Chemotherapy	156			0	0	0	0
Naiyer A. Rizvi (2020) (25)	MYSTIC (NCT02453282)	NSCLC	Durvalumab	369			2	1	0	0
			Durvalumab+ Tremelimumab	371			3	3	3	3
			Chemotherapy	352			0	0	0	0
Robert Jotte (2020) (26)	IMpower131 (NCT02367794)	NSCLC	Atezolizumab+ Chemotherapy	666	3	2				
			Chemotherapy	334	0	0				
Makoto Nishio(2021) (27)	IMpower132 (NCT02657434)	NSCLC	Atezolizumab+ Chemotherapy	291	4	1				
			Chemotherapy	274	2	2				
Yunpeng Yang(2020) (28)	InnovENT (NCT03607539)	NSCLC	Sintilimab+ Chemotherapy	266			8	3		
			Chemotherapy	131			10	0		
Enriqueta Felip(2021) (29)	IMpower010 (NCT02486718)	NSCLC	Atezolizumab+ Chemotherapy	495	2	1				
			Chemotherapy	495	1	1				
L. Gandhi (2018) (30)	KEYNOTE-189 (NCT02578680)	NSCLC	Pembrolizumab +Chemotherapy	405	3	2				
			Chemotherapy	202	0	0				
Howard West (2019) (31)	IMpower130 (NCT02367781)	NSCLC	Atezolizumab+ Chemotherapy	473	2	0	0	0	1	1
			Chemotherapy	232	1	0	1	1	0	0
Luis Paz-Ares (2021) (32)	CheckMate 9LA (NCT03215706)	NSCLC	Nivolumab+ Ipilimumab + Chemotherapy	358	5	4	22	11	26	22
			Chemotherapy	349	0	0	6	0	4	3
Ahmet Sezer (2021) (33)	EMPOWER-Lung 1 (NCT03088540)	NSCLC	Cemiplimab	355			11	1	4	1
			Chemotherapy	342			2	1	0	0
Tony S K Mok (2019) (34)	KEYNOTE-042 (NCT02220894)	NSCLC	Pembrolizumab	636	1	0				
			Chemotherapy	615	0	0				
Z. Wang (2023) (35)	CHOICE (NCT03856422)	NSCLC	Toripalimab+ Chemotherapy	308	3	1	11	0		
			Chemotherapy	156	0	0	1	0		

(Continued)

TABLE 1 Continued

Study (Year)	Trial name (Clinical Trials.gov Identifier)	Type of cancer	Treatment arm	Patient (no.)	Pancreatitis (G1-5)	(G3-5)	AMY (G1-5)	(G3-5)	Lipase (G1-5)	(G3-5)
M. O'Brien (2022) (36)	KEYNOTE-091 (NCT02504372)	NSCLC	Pembrolizumab	580	2	0	2	0	3	0
			Placebo	581	2	1	4	1	2	2
M. Gogishvili (2022) (37)	EMPOWER-Lung 3 (NCT034096614)	NSCLC	Cemiplimab+ Chemotherapy	312	1	0	22	3	15	1
			Chemotherapy	153	0	0	5	0	2	0
G. de Castro (2023) (38)	NEPTUNE (NCT02542293)	NSCLC	Durvalumab+ Tremelimumab	410	2	1				
			Chemotherapy	399	0	0				
S. Peters (2022) (39)	BFAST (NCT03178552)	NSCLC	Atezolizumab	234	2	1				
			Chemotherapy	221	1	0				
Martin Reck (2016) (40)	CA184-156 (NCT01450761)	SCLC	Ipilimumab+Chemotherapy	478			1	0	1	1
			Chemotherapy	476			0	0	0	0
Charles M. Rudin (2020) (41)	KEYNOTE-604 (NCT03066778)	SCLC	Pembrolizumab+ Chemotherapy	223	1	1				
			Chemotherapy	223	0	0				
Jonathan W Goldman (2021) (42)	CASPIAN (NCT03043872)	SCLC	Durvalumab+ Tremelimumab	266	2	1	6	1	10	6
			Durvalumab+ Chemotherapy	265	1	1	11	6	12	9
			Chemotherapy	266	0	0	2	1	7	4
James Larkin (2018) (43)	CheckMate 037 (NCT01721746)	Melanoma	Nivolumab	268	2					
			Chemotherapy	102	0					
Antoni Ribas (2013) (44)	(NCT00257205)	Melanoma	Tremelimumab	328	3	3				
			Chemotherapy	327	0	0				
Ralf Gutzmer (2020) (45)	IMspire150 (NCT02908672)	Melanoma	Atezolizumab+ Vemurafenib + Cobimetinib	230	5	0	46	23	74	47
			Vemurafenib+ Cobimetinib	281	1	0	45	19	77	58
Jeffff rey S Webe (2015) (46)	CheckMate 037 (NCT01721746)	Melanoma	Nivolumab	268						3
			Chemotherapy	102						1
M. B. Atkins (2023) (47)	EA6134 (NCT02224781)	Melanoma	Nivolumab+ Ipilimumab	126	2	1	13	7	18	13
			Dabrafenib+Trametinib	130	0	0	12	1	22	7
G. V. Long (2022) (48)	KEYNOTE-716 (NCT03553836)	Melanoma	Pembrolizumab	487	2	2	3	1	6	4
			Placebo	489	0	0	1	1	8	2
Y.-J. Bang (2018) (49)	JAVELIN Gastric 300 (NCT02625623)	GEJC	Avelumab	184					1	1
			Chemotherapy	177					2	2
Markus Moehler (2020) (50)	JAVELIN Gastric 100 (NCT02625610)	GEJC	Avelumab	243			11	2	9	2
			Chemotherapy	238			9	4	14	7
Kohei Shitara (2020) (51)	KEYNOTE-062 (NCT02494583)	GEJC	Pembrolizumab	254	2					
			Pembrolizumab +Chemotherapy	250	0					
			Chemotherapy	244	1					
Yelena Y Janjigian (2021) (52)	CheckMate 649 (NCT02872116)	GEJC	Nivolumab+ Chemotherapy	782					89	45
			Chemotherapy	767					34	16

(Continued)

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TABLE 1 Continued

Study (Year)	Trial name (Clinical Trials.gov Identifier)	Type of cancer	Treatment arm	Patient (no.)	Pancreatitis (G1-5)	(G3-5)	AMY (G1-5)	(G3-5)	Lipase (G1-5)	(G3-5)
Yoon-Koo Kang (2021) (53)	ATTRACTION-4 (NCT02746796)	GEJC	Nivolumab+ Chemotherapy	359			1	0		
			Chemotherapy	358			4	1		
Kohei Shitara (2018) (54)	KEYNOTE-061 (NCT02370498)	GEJC	Pembrolizumab	294	0	0				
			Chemotherapy	276	1	1				
D.F. Bajorin (2021) (55)	CheckMate 274 (NCT02632409)	UC	Nivolumab	351			33	13	34	18
			Placebo	348			20	5	20	9
Joaquim Bellmunt (2021) (56)	IMvigor010 (NCT02450331)	UC	Atezolizumab	390	2	1	5	2	5	3
			Placebo	397	2	2	0	0	0	0
Thomas Powles (2020) (57)	DANUBE (NCT02516241)	UC	Durvalumab	345	1	0	9	3	11	7
			Durvalumab+ Chemotherapy	340	5	3	12	8	20	16
			Chemotherapy	313	2	1	1	0	2	1
Thomas Powles (2021) (58)	KEYNOTE-361 (NCT02853305)	UC	Pembrolizumab+ Chemotherapy	349	2	2		12		2
			Pembrolizumab	302	2	2		0		0
			Chemotherapy	342	0	0		0		0
R.J. Motzer (2018) (59)	CheckMate 214 (NCT02231749)	RCC	Nivolumab+ Ipilimumab	547					90	56
			Sunitinib	535					58	35
T.K. Choueiri (2021) (60)	CheckMate 9ER (NCT03141177)	RCC	Nivolumab + Cabozantinib	320			47	10	53	20
			Sunitinib	320			29	8	38	15
Thomas Powles(2020) (61)	KEYNOTE-426 (NCT02853331)	RCC	Pembrolizumab+ Axitinib	429	5	4				
			Sunitinib	425	3	3				
S. K. Pal (2022) (62)	IMMOTION-010 (NCT03024996)	RCC	Atezolizumab	390	1	0	4	1	1	1
			Placebo	383	1	1	2	0	3	2
Elizabeth A Mittendorf (2020) (63)	IMpassion031 (NCT03197935)	BC	Atezolizumab+ Chemotherapy	164	0	0				
			Chemotherapy	167	0	0				
Barbara Burtness (2019) (64)	KEYNOTE-048 (NCT02358031)	HNSCC	Pembrolizumab	300	2	0				
			Pembrolizumab+ Chemotherapy	276	1	1				
			Cetuximab + Chemotherapy	287	0	0				
Ezra E W Cohen (2019) (65)	KEYNOTE-040 (NCT02252042)	HNSCC	Pembrolizumab	246			1	1		
			Chemotherapy	234			0	0		
Nancy Y Lee (2021) (66)	JAVELIN Head and Neck 100 (NCT02952586)	HNSCC	Avelumab+ Chemotherapy	348			20	6	10	5
			Chemotherapy	344			5	2	5	1
Eugene D Kwon (2014) (67)	CA184-043 (NCT00861614)	PC	Ipilimumab	393			2	2	2	1
			Placebo	396			1	0	2	2
Zhenggang Ren (2021) (68)	ORIENT-32 (NCT03794440)	HCC	Sintilimab + Bevacizumab	380			2			
			Sorafenib	185			0			
A. L. Cheng (2022) (69)	IMbrave-150 (NCT03434379)	HCC	Atezolizumab+ Bevacizumab	329	10	4				
			Sorafenib	156	6	5				

(Continued)

TABLE 1 Continued

Study (Year)	Trial name (Clinical Trials.gov Identifier)	Type of cancer	Treatment arm	Patient (no.)	Pancreatitis (G1-5)	Pancreatitis (G3-5)	AMY (G1-5)	AMY (G3-5)	Lipase (G1-5)	Lipase (G3-5)
R. K. Kelley (2022) (70)	COSMIC-312 (NCT03755791)	HCC	Atezolizumab+ Cabozantinib	429	4	3	24	3	28	7
			Sorafenib	395	2	0	14	1	14	5
Jing Huang (2020) (71)	ESCORT (NCT03099382)	ESO	Camrelizumab	228	1	1				
			Chemotherapy	220	0	0				
Jong-Mu Sun (2021) (72)	KEYNOTE-590 (NCT03189719)	ESO	Pembrolizumab+ Chemotherapy	370	2	0				
			Chemotherapy	370	1	1				
Kathlen N. Moore (2021) (73)	IMagyn050 (NCT03038100)	OC	Atezolizumab+ Bevacizumab+ Chemotherapy	642	5	4				
			Bevacizumab+ Chemotherapy	644	0	0				
Eric Pujade-Lauraine (2021) (74)	JAVELIN Ovarian 200 (NCT02580058)	OC	Avelumab+ Chemotherapy	182			5	1	3	2
			Avelumab	187			3	0	1	1
			Chemotherapy	177			1	1	0	0
Bradley J Monk (2021) (75)	JAVELIN Ovarian 100 (NCT02718417)	OC	Avelumab+ Chemotherapy	657	1	1	13	4	18	13
			Chemotherapy	334	0	0	4	1	3	2
Cathy Eng (2019) (76)	IMblaze 370 (NCT02788279)	CRC	Atezolizumab+ Cobimetinib	179	2	2	6	3	9	4
			Atezolizumab	90	1	1	2	0	1	1
			Regorafenib	80	0	0	3	0	6	1
D.Reardon (2020) (77)	CheckMate 143 (NCT02017717)	Glioblastoma	Nivolumab	182			3	2	7	4
			Bevacizumab	165			1	0	1	0
Paul Baas (2021) (78)	CheckMate 743 (NCT02899299)	Mesothelioma	Nivolumab+ Ipilimumab	300	2	0	17	7	20	13
			Chemotherapy	284	0	0	1	0	1	1
Dean AFennel (2021) (79)	CONFIRM (NCT03063450)	Mesothelioma	Nivolumab	221	1				1	1
			Placebo	111	0				0	0

AMY, amylase elevation; Lipase, lipase elevation; G1–5, grade1–5; G3–5, grade3–5.

carcinoma (HCC,n=3) (68–70), esophageal carcinoma (ESO,n=2) (71, 72), ovarian cancer (OC,n=3) (73–75), colorectal cancer (CRC,n=1) (76), glioblastoma (n=1) (77) and mesothelioma (n=2) (78, 79). Among the 41 757 patients in the 59 trials that reported information on treatment-related deaths, no pancreatic-related deaths occurred. All included RCTs had a low risk of bias. A detailed evaluation of the risk of bias for each randomized controlled trial is presented in Supplementary Table 2.

Incidence of pancreatic AEs

A total of 41 757 patients were enrolled in the 59 included RCTs (70 ICI-containing arms), including 23 334 (55.9%) patients in the ICI-containing arms and 18 423 patients in the control arms (44.1%). ICI-containing arms included ICI monotherapy in 30/70 arms, ICI plus chemotherapy or targeted therapy in 32/70 arms, and ICI dual therapy in 8/70 arms. In the included 70 arms, NSCLC was the most common tumor type, accounting for 32.9% (23/70), and GEJC accounted for 10.0% (7/70) as the second most common type.

The incidence was 0.93% (95% CI 0.77-1.13, I^2=3.4%) for all-grade pancreatitis and 0.68% (95% CI 0.54-0.85; I^2=0) for grade ≥3 pancreatitis. (Figure 2) Compared with ICI monotherapy, dual-ICI therapy had significantly higher incidences of all-grade pancreatitis (1.10% vs 0.70%) and grade ≥3 pancreatitis (0.94% vs 0.58%) (P< 0.05). (Supplementary Table 3) However, it was not observed in the patients undergoing ICI plus chemotherapy or targeted therapy. An overview of the pancreatitis incidence in different tumor types was shown in Supplementary Table 3. Pancreatitis has a roughly similar incidence in different tumor types (G1-5: 0.30-1.79%, G3-5: 0.17-1.12%).

The incidence was 2.57% (1.83-3.60; I^2=89.2%) for all-grade amylase elevation and 1.17% (0.83-1.64; I^2 =76%) for grade ≥3 amylase elevation. (Figure 2) Compared with ICI monotherapy, dual-ICI therapy had significantly higher incidences of all-grade amylase elevation (3.01% vs 1.66%) and grade ≥3 amylase elevation (1.79% vs 0.78%) (P< 0.05). (Supplementary Table 4)Similar results

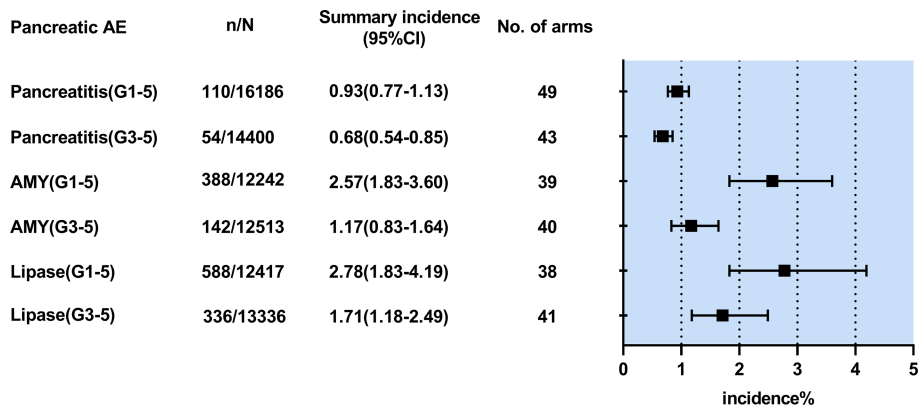

FIGURE 2
Summary pooled incidence analysis of pancreatic adverse events associated with immune checkpoint inhibitor therapy. n/N refers to the number of events (n) observed for the outcome regarding the overall number of patients (N) in patients treated with immune checkpoint inhibitor therapy. AE, adverse event; CI, confidence interval; AMY,amylase elevation;Lipase, lipase elevation;G1-5,grade1-5;G3-5.grade3-5.

were found in the patients treated with ICI plus chemotherapy or targeted therapy (G1-5: 3.78% vs 1.66%, G3-5: 1.57% vs 0.78%, P< 0.05). An overview of the amylase elevation incidence in different treatment regimens and tumor types is shown in Supplementary Table 4. The results showed an increased incidence of all-grade and grade ≥3 amylase elevation in patients with melanoma (5.62%,2.75% respectively) and Mesothelioma (5.67%, 2.33% respectively).

The incidence was 2.78% (1.83-4.19, I^2 =93%) for all-grade lipase elevation and 1.71% (1.18-2.49, I^2 =89%) for grade ≥3 lipase elevation. (Figure 2) Compared with ICI monotherapy, dual-ICI therapy had significantly higher incidences of all-grade lipase elevation (4.08% vs 1.45%) and grade ≥3 lipase elevation (3.28% vs 1.01%) (P< 0.05). (Supplementary Table 5)We found similar outcomes in the patients receiving ICI plus chemotherapy or targeted therapy compared with ICI monotherapy (G1-5: 5.34% vs 1.45%, G3-5: 2.23% vs 1.01%, P< 0.05). An overview of the amylase elevation incidences in different treatment regimens and tumor types is shown in Supplementary Table 5. The patients with melanoma (9.28%,6.14%) are most likely to develop all-grade and grade ≥3 lipase elevation.

Risk of pancreatitis associated with ICI exposure

Pancreatitis as a treatment-related adverse effect was reported in 40 studies (49 ICI-containing arms) and graded using CTCAE. A total of 28 097 patients were evaluated with 16 186 in the ICI-containing arms and 11 911 in the control arms. As shown in Table 2, ICIs significantly increased the risk of all-grade pancreatitis (OR=2.04, 95% CI 1.42-2.94, P = 0.0001; I^2=0) and grade ≥3 pancreatitis (OR=1.90, 95% CI 1.15-3.13, P=0.01; I^2=0). Subgroup analysis suggested that dual-ICI therapy was associated with a higher incidence risk of all-grade pancreatitis (OR=3.47, 95%CI 1.22-9.91, P=0.02). (Supplementary Table 6) A similar statistically significant difference was found in grade ≥3 pancreatitis(OR=3.56, 95%CI 1.09-11.56, P=0.04). Tumor type-stratified analyses showed an increased risk of all-grade pancreatitis(OR=2.55, 95%CI 1.32-4.92, P=0.005) in patients with NSCLC.

Risk of amylase elevation associated with ICI exposure

Amylase elevation as a treatment-related adverse effect was reported in 33 studies (41 ICI-containing arms) and graded using CTCAE. A total of 22 390 patients were evaluated with 12 893 in the ICI-containing arms and 9 497 in the control arms. As shown in Table 2, ICIs significantly increased the risk of all-grade amylase elevation (OR=1.91, 95% CI 1.47-2.49, P < 0.0001; I^2= 29%) and grade ≥3 amylase elevation (OR=2.04, 95% CI 1.46-2.85, P=0.0001; I^2 = 0). Subgroup analysis suggested that all three therapies that

TABLE 2 Summary pooled analysis on the risk of ICI therapy-associated pancreatic adverse events vs. controls in randomized controlled trials.

Variables	Pancreatic AEs							
	Grade 1-5				Grade 3-5			
	OR	95%CI	P	I^2	OR	95%CI	P	I^2
Pancreatitis	2.04	1.42-2.94	P=0.0001	0	1.90	1.15-3.13	P=0.01	0
Amylase Elevation	1.91	1.47-2.49	P<0.0001	29%	2.04	1.46-2.85	P=0.0001	0
Lipase Elevation	1.77	1.37-2.29	P<0.0001	45%	1.89	1.45-2.45	P<0.0001	18%

ICI, immune checkpoint inhibitor; AEs, adverse events; CI, confidence interval; OR odds ratio.

include ICI could significantly increase the incidence risk of all-grade amylase elevation (OR=1.86, 95% CI 1.28-2.69, P=0.001; OR=1.60, 95% CI 1.09-2.35, P=0.02 and OR=3.79, 95% CI 1.68-8.57, P=0.001, respectively). (Supplementary Table 7) Tumor type-stratified analyses showed an increased risk of all-grade amylase elevation in patients with SCLC (OR=4.10,95% CI 1.44-11.63, P=0.008), UC (OR=4.64,95% CI 1.30-16.49, P=0.02), RCC (OR=1.71,95% CI 1.06-2.74, P=0.03), HNSCC (OR=4.00,95% CI 1.55-10.33, P=0.004) and mesothelioma (OR=17.00,95% CI 2.25-128.60, P=0.006).

Risk of lipase elevation associated with ICI exposure

Lipase elevation as a treatment-related adverse effect was reported in 32 studies (40 ICI-containing arms) and graded using CTCAE. A total of 23 461 patients were evaluated with 13 336 in the ICI-containing arms and 10 125 in control arms. As shown in Table 2, ICIs significantly increased the risk of all-grade lipase elevation (OR=1.77, 95% CI 1.37-2.29, P < 0.0001; I^2= 45%) and grade ≥3 lipase elevation (OR=1.89, 95% CI 1.45-2.45, P< 0.0001; I^2 = 18%). Subgroup analysis suggested that both ICI plus chemotherapy or targeted therapy and dual-ICI therapy could significantly increase the incidence risk of all-grade lipase elevation (OR=1.72, 95% CI 1.34-2.20, P<0.0001, and OR=2.92, 95% CI 1.37-6.20, P=0.005 respectively). (Supplementary Table 8) As for grade ≥3 lipase elevation, the trends are similar to those of the all-grade lipase elevation groups. At the same time, we observed a significant increase in the risk of all-grade lipase elevation in the patient with NSCLC (OR=4.23,95% CI 2.14-8.34, P<0.0001), UC (OR=4.20,95% CI 1.46-12.09, P=0.008), RCC (OR=1.53,95% CI 1.16-2.01, P=0.003), and OC (OR=3.42,95% CI 1.17-9.97, P=0.02).

Post-hoc analyses

In this study, we conducted *post-hoc* analyses of PD-1/PD-L1 inhibitors related to pancreatic AEs. As shown in Table 3, the patients with UC undergoing PD-1 inhibitors were at a significantly higher risk of all-grade amylase elevation (OR=5.24,95% CI 2.59-10.57, P<0.0001), all-grade lipase elevation (OR=4.90,95% CI 1.97-12.18, P=0.0006) and grade ≥3 lipase elevation (OR=3.88,95% CI 1.50-10.04, P=0.005), than the patients with UC receiving PD-L1 inhibitors. We conducted *post-hoc* analyses of dual ICI therapy/single ICI therapy-related pancreatic AEs. As shown in Table 4, the patients with NSCLC undergoing dual ICI therapy were at a significantly higher risk of all-grade pancreatitis (OR=4.72,95% CI 1.11-20.17, P=0.04), grade ≥3 pancreatitis (OR= 14.98,95% CI 1.82-123.34, P= 0.01), grade ≥3 amylase elevation (OR=5.95,95% CI 1.30-27.24, P=0.02) and all-grade lipase elevation (OR=4.99,95% CI 1.99-12.55, P=0.0006), than the patients with NSCLC receiving single ICI therapy.

Quality of included studies

Given the significant heterogeneity in the meta-analysis of all the included studies, we performed subgroup analyses to better understand the heterogeneity. (Supplementary Table 9) Some study heterogeneity was suggested by the assessment of all-grade amylase elevation (I^2 = 36%), which appeared to be concentrated in the studies of NSCLC (I^2 = 59%), GJEC (I^2 = 42%) and UC (I^2 = 54%). A similar situation could also be observed with the group of all-grade lipase elevation (I^2=46%) and grade 3 or higher lipase elevation (I^2=26%).

No obvious asymmetry was seen in classic funnel plots, indicating that no evidence of significant publication bias existed. Beyond this, the above view was confirmed by Peter's test. (Supplementary Table 10).

Discussion

In our meta-analysis, we investigated the incidence and risk of pancreatic irAEs associated with ICIs, including pancreatitis, amylase elevation, and lipase elevation. Our findings demonstrated that the incidence of all-grade and grade≥3 pancreatitis with ICIs were 0.93% and 0.68%, respectively. These rates were consistent with previous studies reporting rates of pancreatitis (CTLA-4: 0.9–3%, PD-1: 0.5–1.6%, CTLA4 + PD-1: 1.2–2.1%) (11). Our results also showed that patients treated with

TABLE 3 Odds ratios comparing pancreatic irAEs in patients who received anti-PD-1- vs anti-PD-L1-based therapies.

Cancer	Pancreatitis				Amylase Elevation				Lipase Elevation			
	Grade 1-5		Grade3-5		Grade 1-5		Grade3-5		Grade 1-5		Grade3-5	
	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OP (95%CI)	P
NSCLC	1.25 (0.61-2.55)	0.55	2.15 (0.71-6.52)	0.18	3.05 (0.20-45.65)	0.42	1.57 (0.19-12.78)	0.68	3.47 (0.39-31.17)	0.27	1.81 (0.19-17.47)	0.61
SCLC	1.14 (0.07-18.29)	0.09	1.14 (0.07-18.29)	0.93	–	–	–	–	–	–	–	–
UC	0.84 (0.27-2.56)	0.76	1.45 (0.39-5.32)	0.58	5.24 (2.59-10.57)	<0.0001	1.74 (0.34-8.83)	0.50	4.90 (1.97-12.18)	0.0006	3.88 (1.50-10.04)	0.005

irAEs, immune-related adverse events; OR, odds ratio; CI, confidence interval; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; UC, urothelial carcinoma; Total, pan-cancer.

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TABLE 4 Odds ratios comparing pancreatic irAEs in patients who received dual ICI therapy - vs single ICI therapy -based therapies.

Cancer	Pancreatitis				Amylase Elevation				Lipase Elevation			
	Grade 1-5		Grade3-5		Grade 1-5		Grade3-5		Grade 1-5		Grade3-5	
	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OP (95%CI)	P
NSCLC	4.72 (1.11-20.17)	0.04	14.98 (1.82-123.34)	0.01	2.98 (0.97-9.16)	0.06	5.95 (1.30-27.24)	0.02	4.99 (1.99-12.55)	0.0006	4.91 (0.69-35.02)	0.11

irAEs, immune-related adverse events; OR, odds ratio; CI, confidence interval; NSCLC, non-small cell lung cancer.

dual ICIs therapy had a higher incidence of pancreatitis compared to those treated with monotherapy, and the combination of ICI monotherapy with chemotherapy, targeted therapy, or immunotherapy increased the incidence of pancreatic enzyme elevation. Moreover, our study revealed that melanoma patients had the highest incidence of amylase elevation (G1-5: 5.62%, G3-5: 2.75%) and all-grade and grade 3 or higher lipase elevation (G1-5: 9.28%, G3-5: 6.14%) after receiving immunotherapy.

Our study findings revealed a significant increase in the incidence of pancreatitis, regardless of all grades or grades 3-5, in the ICI group compared to standard chemotherapy or targeted therapy. Further, our subgroup analysis identified a tumor-specific preference for pancreatitis, which was more likely to occur in HCC. Our data suggested that ICI monotherapy did not increase the risk of immune-related pancreatitis, whereas ICI combination therapy did. This may be attributed to the potential of chemotherapeutic agents and targeted drugs to exacerbate pancreatic damage from ICIs. Notably, our data indicated a higher likelihood of pancreatitis in the ICI dual therapy group (G1-5: OR=3.47, 95% CI 1.22-9.91, P=0.02; G3-5: OR=3.56, 95% CI 1.09-11.65, P=0.04). Therefore, additional multi-center RCTs are warranted to confirm its statistical significance. Our results align with previous studies (11, 82, 83).

According to many experts, pancreatitis is more likely to occur in the early stages with low grades, but can be controlled with aggressive intravenous fluid replacement (84, 85). Routine monitoring of amylase and lipase is not recommended for asymptomatic patients unless pancreatitis is clinically suspected (85). However, one study suggests that the use of ICI may increase the risk of developing grade 3 or higher pancreatitis, with clinical symptoms including loss of appetite, vomiting, and abdominal pain (86). Additionally, a case report described a 65-year-old man with stage IV melanoma who developed grade 3 pancreatitis while receiving ipilimumab and pembrolizumab (87). Despite the resolution of clinical signs and symptoms, the patient was diagnosed with pancreatic insufficiency. Interestingly, it seemed that diabetes was also associated with pancreatitis. One study showed that both immune-related pancreatitis and immune-related diabetes occurred earlier than monotherapy when two ICIs were combined, and immune-related diabetes had a later onset than immune-related pancreatitis (88), suggesting that the onset of diabetes might also be a complication of immune-related pancreatitis (89). In order to improve the quality of life and to avoid the long-term sequelae of pancreatitis in patients who have used ICI, vigilant monitoring should be warranted (90).

So far, the exact mechanism of immune-related pancreatitis remains under investigation, and the potential mechanisms may include the increased activity of T cells against antigens present on tumors and normal tissues,the increase in the concentration of pre-existing autoimmune antibodies and the increased levels of inflammatory cytokines (91). Immunohistochemical staining demonstrated a large infiltration of CD3+ T lymphocytes in the non-tumor regions of the pancreas from patients with immune-related pancreatitis (92, 93), which suggested that the potential asscociation of immune-related pancreatitis with autoimmune pancreatitis (AIP) (94). The clinical presentation of AIP differred from that of acute pancreatitis in that abdominal pain and nausea was milder, and positive imaging might be delayed (95).

It is worth noting that despite their widespread use, steroids were not found to be effective in treating immune-related pancreatitis in terms of preventing short- or long-term adverse outcomes, or improving overall survival (84). In fact, exposure to a baseline dose of prednisone equivalent to at least 10 mg/d was found to reduce the efficacy benefit of ICI and significantly shorten progression-free survival (PFS) and overall survival (OS) in NSCLC patients (96). Patients with immune-related pancreatitis were reported to be at risk of relapse upon the resumption of ICI therapy (97). Nonetheless, in general, immunotherapy may be resumed when toxicity returns to grade 1 or lower (85). Our study found that amylase and lipase elevations were more frequent in the ICI group, suggesting a potential immune-related mechanism. Subgroup analyses revealed a significantly higher incidence of all-grade amylase and lipase elevations in melanoma patients. The tumor-specific preference for immune-related elevation of pancreatic enzymes and pancreatitis was similar, with both showing a predilection for NSCLC and UC, as demonstrated by grouping methods based on tumor type or ICI regimen. However, non-specific elevations of pancreatic enzymes due to factors such as alcohol consumption, bowel obstruction, or kidney failure may also occur, leading to a potential overestimation of the incidence of immune-related elevations (98, 99). Nevertheless, unlike pancreatitis, our study provided compelling evidence of a plausible causal association between ICI therapy and elevations of amylase and lipase. We hypothesized that ICI therapy may result in weak pancreatic injury, such as enzyme elevations, rather than robust injury like immune-related pancreatitis. Nonetheless, the decision to continue ICI therapy in patients with grade 3 or higher amylase or lipase elevations without clinical or imaging evidence of pancreatitis after immunotherapy requires further investigation.

It is assumed that the elevation of pancreatic enzymes is associated with pancreatitis and could implicate its development. Research has shown that elevated amylase levels increase the risk of pancreatitis (100). Additionally, 39% of patients with grade 3 or higher lipase elevations had significant clinical symptoms of pancreatitis (84), which was consistent with a retrospective study of 21 cases of immune-related lipase elevations (101). Patients with clinically symptomatic immune-related pancreatitis had higher mean peak serum lipase levels than those without clinical symptoms, but this was not the case in patients with other causes of acute pancreatitis (100). These studies demonstrated that elevated pancreatic enzyme values do not determine the severity of pancreatitis but indicate an increased risk. However, another study found that the true incidence of pancreatitis in patients with immune-related lipase elevations was only 14%, suggesting that in patients with elevated immune-related lipase without clinical symptoms, pancreatic X-ray abnormalities, and diabetes mellitus by fasting blood glucose, the lipase increase may be regarded as a non-clinically significant event (101). Further clinical trials are needed to confirm these findings.

In the *post-hoc* analysis, the findings indicated that PD-1 inhibitors had a significantly higher risk of pancreatic AEs compared to PD-L1 inhibitors, consistent with other immune-related adverse events, such as pneumonitis (15). Furthermore, the study revealed a statistically significant increase in the incidence of pancreatic AEs with dual-ICI therapy relative to single-ICI therapy, possibly due to the similarity in toxicity profiles of CTLA-4 inhibitors and PD-1 inhibitors. In Phase II and III trials of patients with nonresectable melanoma who were randomized to combination versus monotherapy, grade 3 or 4 adverse events occurred in 55–59% of the patients receiving combination therapy, as compared with 16–21% with nivolumab alone and 27–28% with ipilimumab alone (102, 103). Therefore, it is important to be vigilant about the occurrence of irAEs when using dual-ICI therapy, including monitoring pancreatic enzymes.

The study had several limitations. Firstly, our meta-analysis was based on phase III RCTs with strict inclusion criteria, which may limit the generalizability of the findings to real-world settings. Secondly, we may have missed some pancreatic AE cases, as we only analyzed cases recorded in the main text and appendix, which could result in reporting bias (104). Furthermore, some studies included in the analysis were open-label. Thirdly, individual patient data was not available, which prevented us from analyzing the relationship between pancreatic enzyme elevations and pancreatitis or linking immune-related pancreatitis with other irAEs. Lastly, although we acknowledged that drug dose might affect the incidence of irAEs, we were unable to conduct subgroup analyses due to the wide variation in drugs and doses across studies.

Conclusion

Our study offers a comprehensive overview of the incidence and risk of ICI-associated pancreatitis and pancreatic enzyme elevations in various solid tumor types and treatment combinations. Moreover, the *post-hoc* analysis revealed that PD-1 inhibitors have a significantly higher risk of pancreatic AEs than PD-L1 inhibitors, and patients receiving dual ICI therapy have a significantly higher risk of pancreatic AEs than those receiving single ICI therapy. These findings should enhance clinicians' awareness of ICI-associated pancreatic AEs in their clinical practice.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

JL, ZZ, and LP designed the search strategy and confirmed the inclusion criteria. ZZ, WZ, LZ, and SL searched the database, selected the articles, and collected the data. ZZ, LP, WZ, LZ, and SL completed the quality assessment that JL checked. ZZ, LP, WZ, LZ, and SL finished data synthesis and statistics. ZZ write-original draft preparation. JL revised the manuscript carefully. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2023.1166299/full#supplementary-material

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An updated review of gastrointestinal toxicity induced by PD-1 inhibitors: from mechanisms to management

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PD-1 inhibitors, as one of commonly used immune checkpoint inhibitors, enable T-cell activation and prevent immune escape by blocking the PD-1/PD-L1 signaling pathway. They have transformed the treatment landscape for cancer in recent years, due to the advantages of significantly prolonging patients' survival and improving their life quality. However, the ensuing unpredictable immune-related adverse effects (irAEs) plague clinicians, such as colitis and even potentially fatal events like intestinal perforation and obstruction. Therefore, understanding the clinical manifestations and grading criteria, underlying mechanisms, available diverse therapies, accessible biomarkers, and basis for risk stratification is of great importance for the management. Current evidence suggests that irAEs may be a marker of clinical benefit to immunotherapy in patients, so whether to discontinue PD-1 inhibitors after the onset of irAEs and rechallenge after remission of irAEs requires further evaluation of potential risk-reward ratios as well as more data from large-scale prospective studies to fully validate. At the end, the rare gastrointestinal toxicity events caused by PD-1 inhibitors are also sorted out. This review provides a summary of available data on the gastrointestinal toxicity profile caused by PD-1 inhibitors, with the aim of raising clinicians' awareness in daily practice, so that patients can safely benefit from therapy.

KEYWORDS

programmed cell death-1 (PD- 1), immune-related adverse effects (irAEs), colitis, mechanisms, rechallenge, microbiome, biomarkers, novel drug treatment

1 Introduction

Tumors are likely to be the dominant cause of death in the future (1). In response to an upward trend in morbidity and mortality, it is imperative to reduce the global cancer burden gradually and steadily. Compared with the toxicity of chemotherapy drugs, the limitation of surgery timing as well as targetable driver mutations of targeted therapy,

immune checkpoint inhibitors (ICIs) stand out as an emerging immunotherapy approach to combat cancer by regulating the immune function of the organism and tumor microenvironment (TME). Programmed cell death-1 (PD-1) inhibitors as a type of ICIs are designed to boost T-cell activation and levels of proinflammatory cytokines along with concentrations of autoimmune antibodies against cancer by blocking PD-1 protein expression (2). Accordingly, it opens an avenue of tumor treatment and rapidly expanded to first-line settings for its powerful clinical efficacy on avoiding immune escape of tumor cells (3).

Despite ongoing progress in PD-1 monoclonal antibodies including nivolumab, pembrolizumab, and sintilimab, the disrupted balance of immune tolerance and systemic inflammatory reactions in a seemingly unpredictable fashion will result in organ-specific toxicities. Such immunogenic adverse events that occur during or after ICI therapies are described as immune-related adverse events (irAEs) (4).

Colon inflammation (colitis), with or without small bowel inflammation (enterocolitis), are the dominant adverse events associated with anti–PD-1 therapy, manifested as abdominal pain, diarrhea, blood, and mucus in stools (5). The incidence of diarrhea was reported to be 12.1–13.7%, and the incidence of colitis was 0.7–1.6% in patients with anti–PD-1 (6). In addition, an increasing number of rare and potentially life-threatening irAEs are being reported such as bowel perforation or obstruction. A meta-analysis involving 19,217 oncology patients demonstrated that fatal toxic effects induced by anti–PD-1 agents occur at a rate of 0.36%, with death from colitis accounting for approximately 0.066% (7). The median time to onset of gastrointestinal adverse events was about 40 days and, to ICIs-related, fatality was about 43 days after treatment (7, 8). Several patients opt for combined anti–PD-1 and anti-cytotoxic T lymphocyte antigen 4 (CTLA-4) to achieve a more satisfactory efficacy, but it is accompanied by more and faster occurrence of adverse events (9), of which pembrolizumab plus ipilimumab had the shortest median time to onset irAEs (10).

Unexpectedly, adverse events imply better outcomes. More extensive endoscopic inflammation rather than being limited to the left colon, acute histological inflammation, higher grade colitis, and recurrent diarrhea tend to have better long-term survival outcomes (11). There is no doubt that high-grade irAEs account for temporary or permanent discontinuation of immunotherapy. Up to 3–12% of patients forgo further anti–PD-1 therapy, although most irAEs can be ameliorated by symptomatic therapy with (or without) corticosteroids (6, 12). Given that risk and benefit may affect the patients' life quality, the question of whether to restart ICI therapy after adverse events has become a dilemma for clinicians.

Immune cells and inflammatory factors are indispensable in the progression of irAEs, and even specific strains of gut microbes are mechanistically linked to susceptibility of irAEs. For instance, the phylum *Bacteroidetes* is associated with a lower incidence of immune-associated colitis, whereas the phylum *Firmicutes* is associated with a higher incidence of colitis (13). Despite continuous exploration in recent years, the pathogenesis of toxicity is not well clarified. The high prevalence has led to classification method and empirical management guidelines being introduced and updated. However, standardized irAEs guidelines are not adequate enough for lack of a wealth of experience and high-quality evidence. Consequently, it is crucial to focus on biomarkers, establish risk stratification models, and perform routine testing or biopsies if necessary (5, 14).

In this review, gastrointestinal side effects after anti–PD-1 therapy are in the spotlight. By comprehensively summarizing relevant mechanisms, enriched therapeutic arsenal, potential predictors and risk factors, we hope to provide a theoretical basis and shed light on novel therapeutic strategies in PD-1–mediated irAEs that address toxicity without eliminating antitumor efficacy, allowing patients to safely benefit from treatment.

2 Clinical manifestations and grading criteria

Diarrhea is a disorder characterized by an increase in frequency and/or loose or watery bowel movements. Due to intestinal inflammation, colitis often manifests as diarrhea, abdominal pain, distension, blood or mucus in the stool, fever and even upper gastrointestinal symptoms like nausea and vomiting.

Clinicians typically choose endoscopy with biopsy to assist in verification when they find suspected patients. Colitis has a range of presentations on endoscopy, including ulcerations or non-ulcerative inflammatory morphology such as diffuse or patchy erythema, inflammatory exudate, loss of vascular pattern, aphthae, edema, friability, erosions or granular mucosa. The histological features are generally similar to acute colitis, manifested as intestinal lamina propria expansion, intraepithelial neutrophilia and neutrophil crypt abscess; but may also present as chronic inflammation, described as basal lymphocytic infiltrate, cryptic architecture distortion, and Paneth cell metaplasia (11). Patients with severe colitis symptoms need to be alert for complications like bowel perforation and intestinal obstruction, and CT scan can be used to evaluate bowel wall thickening, colonic distension, mesenteric vessel engorgement, abscess, and perforation (2).

Immune-related toxicities were graded by based on Common Terminology Criteria for Adverse Events (CTCAE) v5.0. For details, check the table below. Grade 1 (G1) diarrhea is defined as an increase of less than four bowel movements per day as well as a mild increase in ostomy output compared with baseline. G1 colitis is often asymptomatic but detectable by imaging changes. G2 diarrhea is defined as an increase of 4-6 bowel movements per day, moderate increase in ostomy output over baseline, and limited instrumental activities of daily living (ADLs). Patients with G2 colitis suffer from mild or moderate abdominal pain and mucus or blood in stool. G3 diarrhea is defined as more than seven bowel movements per day, severe increase in ostomy output from baseline as well as limited self-care ADLs such as bathing, feeding and taking medications, and the involved patients usually require hospitalization. G3 colitis often presents with severe abdominal pain and peritoneal signs. G4 diarrhea/colitis may be combined with life-threatening consequences such as perforation, bleeding or toxic megacolon that require urgent intervention. All G5 diarrhea/colitis patient outcomes are defined as death (Table 1).

TABLE 1 Grading of irAEs referring to the CTCAE v5.0.

Gastrointestinal disorders	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Diarrhea	Increase of less than 4 stools per day and mild increase in ostomy output compared with baseline.	Increase of 4–6 stools per day and moderate increase in ostomy output compared with baseline; limited instrumental ADLs.	Increase of more than 7 stools per day and severe increase in ostomy output compared with baseline; limited self-care ADL such as bathing, feeding and taking medications; hospitalization indicated.	Life-threatening consequences such as perforation, bleeding or toxic megacolon; urgent intervention indicated.	Death
Colitis	No clinical symptoms, but imaging changes may be present.	Mild or moderate abdominal pain as well as mucus or blood in stool.	Severe abdominal pain and peritoneal signs.	Life-threatening consequences such as perforation, bleeding or toxic megacolon; urgent intervention indicated.	Death

3 The underlying mechanisms

Most scholars are in favor of viewpoints that the occurrence of irAEs is regulated by a subclinical or latent autoimmune state and patients tend to develop the corresponding clinical symptoms after ICI treatment (15). In that case, what is the pathogenesis of irAEs actually? It is still under continuous investigation, but basically revolves around infiltration by T lymphocytes and innate lymphocytes, the cytokine storm, as well as B lymphocytes-mediated elevation of autoimmune antibody concentrations. Clarifying the immune pathways involved in irAEs will assist in the development of therapeutic modalities that can prevent or mitigate irAEs without compromising antitumor immunity.

3.1 Changes in immune cell profile

3.1.1 Bulk T-cell receptor diversity

Immune cells such as T cells can express PD-1, and when PD-1 binds to PD-L1 ligands expressed by cytokine-stimulated tumor cells, it can aggregate with T-cell receptor (TCR) and induce the dephosphorylation of the proximal TCR signaling molecules, thereby inhibiting T-cell activation. PD-1 inhibitors are intended to enhance T-cell anti-tumor response by blocking the above process (13).

Andrews and colleagues evaluated the immune profile of patients' peripheral blood and concluded that high-grade irAE was strongly tied to increased TCR diversity and enhanced T-cell expansion at baseline (16). Likewise, Jing et al. proposed that TCR diversity and $CD8^+$ T-cell abundance showed the greatest correlation with irAE (17).

To further understand the mechanisms involved, Lozano et al. collected peripheral blood samples from metastatic melanoma patients treated with anti–PD-1 monotherapy or combination ICIs of anti–PD-1 and anti–CTLA-4, and built a strong connection between severe irAEs within 3 months of treatment initiation and pre-treatment circulating activated $CD4^+$ memory T-cell abundance as well as bulk TCR diversity (15). In addition, a greater magnitude of TCR clonal expansion may contribute to more rapid progression of severe irAEs (15). Regrettably, the study lacks sufficient evidence for delayed irAEs beyond 3 months; in addition, it needs to determine whether the conclusions drawn in metastatic melanoma can be generalized to other tumor types by prospectively in-depth validation.

3.1.2 The T helper 1-skewed phenotype

T-box expressed in T cells (T-bet) was identified as the master regulator for the differentiation of T helper 1 (Th1) cells. Its expression is significantly associated with prognosis, attributed to the promotion of Th1 cell differentiation. In human tumor tissues, when T-bet is lowly expressed, the Th1/Th2 balance is disrupted and tilted toward Th2 cells and tumor immune escape occurs; in contrast, high expression of T-bet promotes Th1 cell differentiation and exerts significant anti-tumor effects, which may predict a positive prognosis (18, 19).

After summarizing two cases of nivolumab-induced severe colitis, Yoshino et al. proposed that the mechanism of their adverse events had greater likelihood of a dominant response of Th1 cells, demonstrated as a strong infiltration of $CD4^+$ cells expressing T-bet (20). When compared intestinal samples with irAEs colitis to healthy intestinal samples, Reschke et al. noticed an expansion of the Th1/Tc1-type cytokine profile of Tissue-resident memory T cells (T_{RM}) in irAEs colitis, with increased expression of IL-15 required for their differentiation and survival (21). Markedly upregulated TNF-α; IFN-γ secreted by Th1/Tc1; and chemokines such as CXCL9, CXCL10, CXCL11, and dominant expression of checkpoint receptors (e.g., PD-1 and CTLA-4) were also detected. Interestingly, the adhesion molecule ITGA4, which is hardly expressed in healthy colon, was found a growing number in irAEs colon samples (21).

As a result, it is reasonable to speculate the Th1-skewed phenotype is of potential mechanisms driving the correlation between irAE and antitumor response

3.1.3 Reactivation of effector T cells

Blocking the PD-1 signaling pathway with anti–PD-1 agents reactivates exhausted effector T cells to kill tumor cells, and the autoantigens released by tumor lysis contribute to autoimmunity. In addition, that is exactly what happened; the occurrence of irAEs was accompanied by high levels of $CD4^+$ and $CD8^+$ effector memory T cells (22). PD-1 inhibitors cause hyper-responsiveness cytotoxic T lymphocytes (CTLs) and Th1 cells in irAEs, with excessive

production of interferon γ (IFN-γ), granzyme B (GZMB), interleukin 12 (IL-12), and tumor necrosis factor α (TNF-α) (23).

Tissue-resident memory T cells (T_{RM}), a class of uncirculated lymphocytes persist in peripheral tissues, are the initial mainstays defending against local infections and play a key role in tumor immunotherapy and autoimmune diseases (24). By single-cell RNA sequencing (scRNA-seq) and flow cytometry, Luoma et al. (25) first analyzed immune cell changes at the single-cell level in intestinal biopsy samples from melanoma patients with ICI-induced colitis, revealing that cytotoxic T cells and proliferating T cells derived from T_{RM} were enriched in colitis samples, with high expression of IFN-γ and GZMB. Since T_{RM} is already abundant in healthy colon, colitis may be early irAEs attributed to the rapid activation of T_{RM} after recruitment of T cells from the blood.

3.1.4 Regulatory T cells

Regulatory T cells (Tregs) modulate the formation of an immune-suppressive microenvironment and promote tumor immune evasion. Conflicting opinions exist in regards to the role Tregs play in ICI-induced colitis.

Anti-CTLA-4–mediated antitumor immunity and irAEs are attributed to Tregs depletion (23). Luoma and colleagues discovered that Treg cluster 1 cells were concentrated on colitis caused by anti–CTLA-4 monotherapy or in combination with anti–PD-1, expressing some genes specific to Th1 cells, such as IL-12 receptor and CXCR3, therefore revealing insufficient evidence of regulatory T-cell depletion in colitis (25). However, the role of Tregs in anti–PD-1 monotherapy-mediated colitis deserves more research to prove.

3.1.5 Elevation level of innate lymphocytes

Innate lymphocytes (ILCs) are widely distributed in the human bodies, especially in intestinal mucosal tissues, and group 3 innate lymphoid cells (ILC3) are responsible for intestinal homeostasis by secreting IL-22, IL-17 and GM-CSF (26).

A growing body of clinical evidence suggests that some patients develop hyperprogressive disease (HPD) with accelerated tumor growth after anti-PD1 immunotherapy. Probing the mechanism revealed that the ILC3s are activated and upregulated in these patients, helping to suppress T cells responses and inducing T cells death, which hastens disease progression and leads to poor prognosis (27).

Wang et al. observed in tumor-bearing mice a correlation between the severity of immune-induced colitis and the increased number of ILC3s in their intestinal mucosa, regardless of ILC1 and ILC2; reduction of the mucosal number of ILC3s improved the colitis symptoms, and the inflammatory indicators such as IL-17 showed a tendency to decrease to normalization (28).

In general, perhaps as a result of T cell reactivation, inhibition of ILC3 reduces the risk of developing HPD after treatment, in parallel with reducing the development of ICI-related adverse effects such as colitis. As a matter of concern, however, it was proposed that a decrease in ILC3 may drive resistance to ICI therapy in colorectal cancer patients (29). More in-depth exploration of ILC3's potential contribution to immunotherapy is warranted, but in any case, its powerful immunomodulatory properties hold great promise as a target for cancer therapy.

3.1.6 B cells activation and autoantibodies

B-cell activation and subsequent autoantibody production play a considerable role in the development of irAEs. However, the role of B cells in antitumor immunity is still controversial.

The percentage and absolute number of B cells decreased with irAEs and the magnitude of the decrease was strongly positively correlated with the severity of the irAE, enabling early identification of patients at risk of toxicity through prophylactic monitoring of B cells (22, 30). A decline in circulating B cells and an increase in CD21lo B cells and plasmablasts are detectable in the first cycle after ICI treatment. Of note, patients with early alterations in the B cell populations were more likely to develop grade 3 or higher irAEs by the 6th month of ICI use, with these changes preceding toxicity by a median of 3 weeks (31).

CXCL13, known as B-lymphocyte chemokine ligand, regulates the homing of B cells to lymphoid follicles by binding to the receptor CXCR5. Anti-PD-1–induced irAEs were followed by upregulated plasma expression of CXCL13 (32). Another team observed that irAEs lead to the accumulation and dysfunction of $CXCR5^+$ invigorated T follicular helper cells (Tfhs) in the germinal centers, ultimately leading to B cell-mediated autoantibody overproduction (23).

Removal of B cells from the mice revealed a decrease in circulating immunoglobulin G (IgG) levels, which were previously elevated, as well as alleviated organ tissue damage (32). Anti–PD-1 therapy triggers B-cell–mediated antibody-dependent irAEs and IgG levels in baseline serum may predict the development of irAE (33). More than that, one prospective study evaluated the association of baseline serum IgG and its subclasses levels with antitumor response and survival after ICI therapy in 49 patients, revealing that high-rise levels of total IgG (> 9.66 g/liter, $P = 0.038$), IgG1 (> 6.22 g/liter, $P = 0.025$), IgG2 (> 2.42 g/liter, $P = 0.019$), and IgG3 (> 0.21 g/liter, $P = 0.034$) were significantly and positively correlated with PFS, whereas OS prolongation was only notably relevant with elevated IgG2 subclasses (34).

Additionally, measurement of pre-treatment circulating autoantibodies in melanoma patients treated with ICI revealed that elevated anti-MAGEB4 levels were linked to longer OS and development of irAEs, while high levels of pre-treatment anti-FGFR1 antibodies were linked to shorter OS and lower frequency of irAEs (35).

3.2 Inflammation storm

Not only the changes in the immune cell profile, but also the inflammatory storm caused by systemically activated pro-inflammatory cytokines. Eleven circulating cytokines, known as G-CSF, GM-CSF, Fractalkine, FGF-2, IFN-α2, IL-12p70, IL-1a, IL-1B, IL-1RA, IL-2, and IL-13, were dramatically upregulated in

melanoma patients with severe irAE at baseline and early during combined ICI treatment, and were integrated into a new cytokine toxicity score (36).

3.2.1 IL-1

A higher expression profile of the NLRP3 inflammasome was observed in bone marrow cells of patients with immune-associated colitis, whose activation triggers IL-1 release, suggesting that IL-1 may be involved in the development of colonic inflammation (25).

With combined blockade of CTLA-4 and PD-1, colitis-related symptoms such as diarrhea and weight loss were not identified by Andrews et al. (16) in the mouse model, but subclinical toxicity like shortened ileal villi, mucosal damage and inflammatory infiltration were uncovered. Transcriptional analysis of the ileum verified rapid Transcriptional upregulation of IL-1b, rather than TNF-α or IL-6. After tentatively addition of IL-1R antagonist, the intestinal inflammation was alleviated noticeably, hinting the gut microbiome mediates ICI-induced intestinal toxicity via IL-1β. The specific mechanisms underlying this are, as yet, unclear.

3.2.2 IL-6

IL-6 is a key factor in the differentiation of $CD4^+$ T cells into Th17. IL-6-induced inflammation was observed in patients who developed immune-related enterocolitis (irEC) after treatment with ICI, as evidenced by more incredible upregulation than normal tissue of acute phase reactants (e.g. IL-11), genes encoding neutrophil and monocyte chemotactic molecules (e.g. CXCL1, CXCL2, CXCL3 and CXCL8) and neutrophil count (37). Surprisingly, there was no apparent elevation of Th17 memory cells in patients treated with anti–PD-1 therapy, while completely different from anti–CTLA-4 therapy with abundant Th17. Therefore, inflammation mediated by IL-6–Th17 pathway can probably drive the progress of irEC, so that addition of IL-6 blockers to tumor-bearing mice treated with anti–CTLA-4 reduces Th17, macrophages counts and tumor load, but promotes tumor shrinkage and increases survival rate of mice (37).

3.2.3 IFN signaling pathway

T cell – myeloid crosstalk relies on the IFN signaling pathway.

IFN-γ induces hyper-expression of CXCL9, CXCL10 (ligand for CXCR3) and CXCL16 (ligand for CXCR6) in bone marrow cells of patients with colitis, which then encodes chemokines that recruit effector T cells to the site of inflammation; accordingly, expression of chemokine receptor genes CXCR3 and CXCR6 is upregulated in the T cell population (25). The molecular mechanism of T cell recruitment may be a therapeutic target, for example, blockade of CXCR3 or CXCR6 could theoretically ameliorate cancer metastasis and reduce intestinal inflammation (Figure 1).

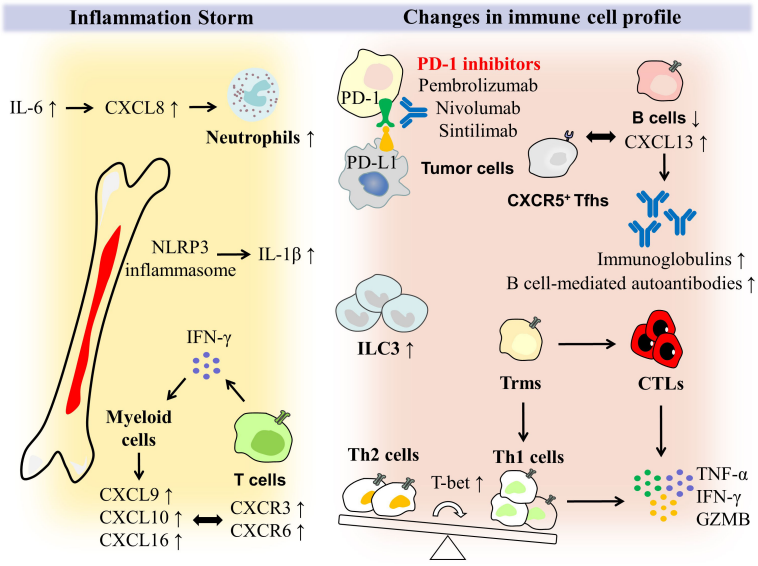

FIGURE 1
Potential mechanisms of immune cells and inflammatory storm in PD-1 induced colitis. PD-1 expressed by T cells can bind to PD-L1 ligands activated by tumor cells, thereby inhibiting T cell activation. PD-1 inhibitors such as pembrolizumab, nivolumab and sintilimab block these processes to enhance the T cell anti-tumor response. With high expression of T-bet, the balance of $CD4^+$ T cells is tilted toward Th1 cells, which is dominant compared with Th2 cells. Moreover, tissue-resident memory T cells (Trms) rapidly differentiate into CTLs and Th1 cells, and secrete the pro-inflammatory factors like TNF-α, IFN-γ and GZMB. ILC3 was enriched in intestinal mucosal tissues. As irAEs occur and grow in severity, the absolute number of B cells decreases. However, CXCR5+ T follicular helper cells (Tfhs) accumulate in the germinal centers and plasma expression of the B lymphocyte chemokine ligand CXCL13 increases, ultimately allowing for B cell-mediated overproduction of autoantibodies and immunoglobulins. In PD-1-induced colitis, there is not only a change in the immune cell profile, but also an onslaught of inflammatory storms. NLRP3 inflammasome expression and induction of IL-1β release are found to be higher in myeloid cells. IL-6 induces an increase in CXCL8, which encodes a neutrophil chemotactic molecule, giving rise to the number of neutrophils. IFN-γ plays an essential role in the interaction between T cells and myeloid cells, as it stimulates high expression of CXCL9, CXCL10, and CXCL16 in myeloid cells, allowing T cell populations expressing CXCR3 and CXCR6 chemokine receptors to be rapidly recruited to inflamed tissues and secrete IFN-γ, which eventually formed a closed loop of the IFN signaling pathway.

3.3 The role of gut microbes

In patients treated with anti–CTLA-4, their baseline gut microbiota was found to be associated with an elevated susceptibility to ICI antitumor response and to the development of enterocolitis (6). Although anti–PD-1 drugs have milder gastrointestinal irAEs compared with anti–CTLA-4 (9), a growing attention has been paid to the gut microbiome of patients treated with PD-1 inhibitors. How the microbiota and their metabolites affect anti–PD-1 efficacy and gastrointestinal adverse outcomes is gradually being unveiled.

3.3.1 Changes in the gut microbial spectrum

Scholars have identified a lot of overlap with microorganisms that are highly responsive to immunotherapy and those involved in prevention of irAE development. Some bacterial species such as *Akkermansia muciniphila* (*A. muciniphila*), *Bacteroides fragilis*、*Bifidobacterium* spp., *Faecalibacterium* spp. (especially *F. prausnitzii*) and *Ruminococcaceae* spp.are involved in favorable antitumor immune responses (38, 39). Patients who responded poorly to ICI treatment and suffered from grade 3 or higher severe adverse reactions had significantly lower microbiome diversity at baseline (39). Therein, declined relative abundance of *A. muciniphila*, *F. prausnitzii* and *Ruminococcaceae* family was observed in the intestine of patients with severe irAE (39). *Bacteroides intestinalis* and *Intestinibacter bartlettii* were observed to be enriched in melanoma patients who developed grade 3 and higher irAEs; while *Anaerotignum lactatifermentans* and *Dorea formicigenerans* were enriched in patients with low-grade toxicity (16). Likewise, Zhang et al. (40) analyzed the gut microbiota profiles of 23 patients with gastrointestinal cancer who received immunotherapy recently, finding that species such as *Clostridium hathewayi*, *Ruminococcus torques* and *Megamonas* were enriched in patients without irAEs; inversely, *Bifidobacterium dentium*, *Rothia mucilaginosa* and *Gemella haemolysans* were significantly higher in irAE patients. Moreover, *Ruminococcus callidus* and *Bacteroides xylanisolvens* were enriched in patients without severe irAEs, which helps to distinguish the population with ≥3 irAE.

Assessment of fecal microbiota composition by 16S rRNA gene sequencing revealed that the abundance of the phylum *Bacteroidetes* was negatively associated with anti-CTLA4–associated colitis, in which patients enriched are less likely to develop colitis perhaps due to expression of polysaccharide A can induce Tregs to prevent colitis; while patients enriched in *Faecalibacterium* genus and phylum *Firmicutes* are more likely to develop colitis, although with longer PFS and OS (23, 41). In the same way, stool samples from lung cancer patients were tested and *Raoultella* were discovered to be overrepresented in the feces of patients who undergo less serious irAE; whereas *Agathobacter* was linked to more severe irAE profile, although it involved in beneficial clinical outcomes (42).

Of concern, the classic probiotics *Lactobacillus* and *Bifidobacterium* play an important role. Experiments by Wang and companions demonstrated a significant decrease in the relative abundance of *Lactobacillus* in severe ICI-associated colitis. After complete clearance of *Lactobacillus* with antibiotic vancomycin, the mice immediately displayed obvious weight loss, severe inflammatory cell infiltration and elevated levels of pro-inflammatory cytokines such as TNF-α, IL-6 and IFN-γ. Instead, all these manifestations were noticeably improved via reducing in ILC3s after oral administration of probiotic *Lactobacillus reuteri* adequately supplemented with *Lactobacillus*, defined as no further weight loss, less inflammatory cell infiltration, and partial restoration of colonic structure in mice. It is noteworthy that there was no visible difference in the count of tumor-infiltrating T cells and the growth rate of tumors as well as the OS of mice was not affected. In summary, they highlight the intestinal microorganism *Lactobacillus* can symptomatically attenuate ICI-induced immune-mediated colitis without affecting the efficacy (28). It is quite remarkable that Sun et al. (43) revealed *Bifidobacterium*, one of the well-known probiotics, was able to induce microbiome optimization, such as an increase in the proportion of *Lactobacillus*, after colonizing the gut of mice. This altered commensal community enhanced the IL-10–mediated inhibitory function of intestinal Tregs, which contributed to the mitigation of colitis in mice in the context of CTLA-4 blockade. When dual immune checkpoint blockade was performed, Tan et al. discovered *Lactobacillus rhamnosus GG* alleviated severity of irAEs colitis in mouse models, as manifested by significantly reduced disease activity index, histopathological score and CD8+ T cell counts, together with increased FoxP3$^+$ Tregs (44).

Perhaps other technologies such as macrogenome sequencing can be applied in the future to better characterize the entire gut microbiome and clarify its relationship between clinical benefit and adverse effects of ICI treatment.

3.3.2 Metabolites of intestinal flora

Accumulating studies have led scholars to speculate that the poor outcome of immunotherapy is connected to the decrease in beneficial microbiome metabolites, including B-vitamins synthesis, inosine as well as short chain fatty acid (SCFA) production (39).

3.3.2.1 B-vitamins

The gut microbiota produces bioactive compounds including B-vitamins, which have been reported to act not only as nutrients but also as modulators of colitis (45). For instance, vitamin B3 (niacin) deficiency leads to intestinal inflammation and diarrhea, while proper supplementation with vitamin B6 can alleviate IBD (46).

3.3.2.2 Bacterial-derived metabolite inosine

Following the action of ICIs, the metabolite inosine produced by *A. muciniphila* and *B. pseudolongum* promotes the activation of anti-tumor Th1 cells through T-cell–specific A_{2A} receptor signaling in response to ICIs immunotherapy (47). Hereafter, the efficacy of the metabolism and recovery of inosine degradation products including xanthine and hypoxanthine in immunotherapy deserves further study.

3.3.2.3 Short chain fatty acid

SCFA includes propionate and butyrate. Propionate and butyrate can improve intestinal barrier function, accelerate the repair of intestinal epithelial cell damage and maintain intestinal homeostasis (48, 49). In the analysis of gut microorganisms from patients' fecal

samples with gastrointestinal cancers treated with anti–PD-1/PD-L1 therapy, there were overrepresented commensal SCFAs-producing bacteria, including *Eubacterium*, *Lactobacillus*, and *Streptococcus* in patients with satisfactory outcomes (50). Zhang et al. observed an enrichment of *Eubacterium rectale* and *Megasphaera elsdenii*, in non-/low irAEs gastric cancer patients (40).

SCFA is the main fermentation product of dietary fiber. Turning our attention to eating habits, dietary fiber intake affects the immune function of the gut microbiota and the development of irAEs. Propionate levels are significantly elevated in melanoma mice on a fiber-rich diet (51). In the case of high abundance of butyrate-producing *Ruminococcaceae*, researchers found that the corresponding hosts positively responded to dietary nutrient intake, i.e., displayed high fiber and omega 3 fatty acid consumptions (39). Consequently, a high dietary can be responsible for increase in *Ruminococcaceae* to support the maintenance of intestinal integrity; on the contrary, a low dietary is easily susceptible to poor response and adverse outcomes in immunotherapy.

When Spencer et al. evaluated melanoma patients treated with anti–PD-1, they revealed that patients with adequate dietary fiber intake had a high microbial alpha diversity with abundant *Ruminococcaceae* family and *Faecalibacterium* genus as well as a prolonged PFS, even if not statistically significant. Subsequently, they performed corresponding parallel animal experiments. Melanoma mice with anti–PD-1 therapy exhibited tumors delayed growth after a high-fiber diet, accompanied by increased $CD4^+$ T cells; in contrast, mice receiving a low-fiber diet had significantly fewer IFN-γ^+ $CD8^+$ T cells for mice with impaired treatment response to anti-PD-1–based therapy. It should be emphasized that none of these results could be observed in germ-free mice. It is suggested that dietary intervention for tumor immunotherapy is gut microbial-dependent and that fiber contribute to the immunotherapy by gut flora affecting T cell activation. Therefore, we urge oncology patients with PD-1 inhibitors therapy to pay more attention to dietary habits in daily life (51) (Figure 2).

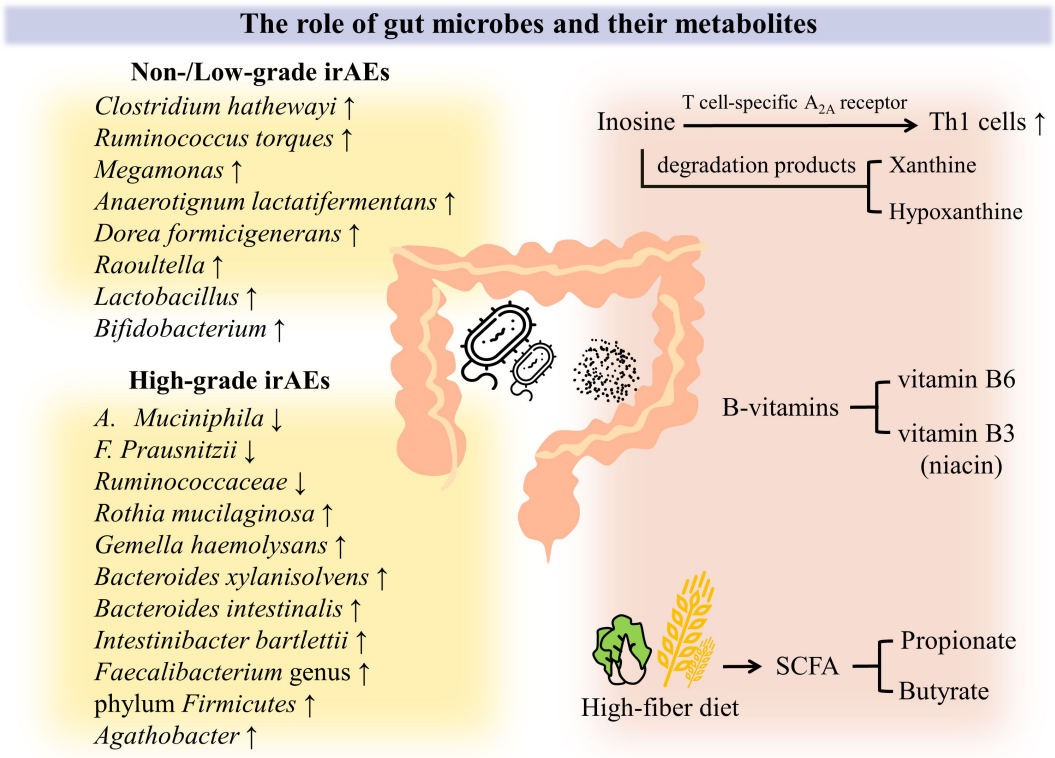

FIGURE 2
Crosstalk between gut microbiome and PD-1 induced colitis. A growing body of evidence demonstrates that intestinal flora and its metabolites play an influential role in the occurrence and varying severity of irAEs. First of all, the intestinal microbial spectrum has changed. Even with the heterogeneity of tumor types and dosing regimens, Clostridium hathewayi, Ruminococcus torques, Megamonas, Anaerotignum lactatifermentans, Dorea formicigenerans, Raoultella, Lactobacillus and Bifidobacterium was found to be abundant in non-/low-grade irAEs patients. When it comes to high-grade irAEs, declined abundance of A. muciniphila, F. prausnitzii, Ruminococcaceae was observed, but Rothia mucilaginosa, Gemella haemolysans, Bacteroides xylanisolvens, Bacteroides intestinalis, Intestinibacter bartlettii, Faecalibacterium genus and phylum Firmicutes, Agathobacter was found to be enriched. In addition, the role of intestinal flora metabolites has been increasingly appreciated. B vitamins such as vitamin B3 and B6 can alleviate intestinal inflammation to some extent. Inosine enhances the antitumor effect by promoting Th1 cells activation through T-cell–specific A2A receptor, and even its degradation products xanthine and hypoxanthine may play a role. Gut microbial-dependent dietary interventions are currently emphasized, as high dietary fiber promotes an increase in the proportion of SCFA-producing bacteria, resulting in significantly higher levels of SCFA (e.g., propionate and butyrate), which can effectively reduce intestinal inflammation and maintain intestinal homeostasis.

4 Treatment strategies

4.1 Pre-treatment preparation

Intestinal inflammatory markers such as fecal lactoferrin and calprotectin should be performed as part of the initial workup, which is critical to determine disease activity and guide therapeutic intervention. Meanwhile, patients with G2 and higher grades diarrhea/colitis symptoms should undergo fecal infectivity analysis to rule out infections such as *Clostridium difficile* and cytomegalovirus (CMV) in addition to whole blood PCR for routine blood count and serum CRP. Clinical suspicion of celiac disease was clarified by serum anti-tissue transglutaminase immunoglobulin (tTG-IgA).

Approximately 95% of patients have ICI-induced inflammation in the left colon on biopsy (52). Recently, a cross-sectional study organized by De Silva et al. uncovered 93.8% of left-sided colon (defined as distal 25 cm of the colon, rectosigmoid colon) biopsies with an abnormal endoscopic appearance and, to the surprise, up to 68.6% of normal-appearing mucosal biopsies with histological evidence of colitis (53). They finalize by noting that extensive biopsies of left-sided normal and abnormal segmental mucosa for evaluation of immune-mediated colitis with flexible sigmoidoscopy hold promise as a simplified alternative to full colonoscopy, although poor sensitive but extremely specific and is often sufficient for early initial diagnosis or follow-up review to guide treatment strategy. Thus, flexible sigmoidoscopy or colonoscopy with biopsy is strongly recommended within 2 weeks of onset for all G2patients and G1patients with positive lactoferrin.

4.2 Symptomatic treatment

For patients with G1 diarrhea/colitis, in case of a negative lactoferrin result and no infection, symptomatic supportive management including a low-fiber dietary combined with the antidiarrheal drugs such as loperamide, if necessary, mesalamine or cholestyramine may be administered.

4.3 Corticosteroid

The cornerstone of toxicity management is often immunosuppressive agents, which is widely used as a first-line treatment. However, the side effects of their long-term use have been demonstrated such as adrenal insufficiency and osteoporosis, particularly the increased risk of opportunistic infections (54), and even high dose of glucocorticoid (≥ 60 mg prednisone equivalent once a day) in early onset severe irAEs (within 8 weeks of anti–PD-1 initiation) has recently been implicated in compromising the antitumor efficacy of PD-1 inhibitors, resulting in poorer PFS and OS in patients (55). In view of its double-edged sword effect, the use of hormones should be individualized after a comprehensive assessment of patient's responsiveness and tolerance. Pay attention to the past medical history of patients, preferably the elderly with diabetes or in an immunocompromised state (54). Use the lowest effective dose of corticosteroids within the shortest possible time.

To optimize follow-up management strategies, it is imperative to focus on endoscopic and histologic features for their early suggestive role. Wang et al. elucidated that, in patients with endoscopically ulcers, there was a clinically higher frequency of steroid-refractory colitis ($p = 0.044$) and severe diarrhea ($p = 0.033$) (11). In light of this finding, clinicians may administer stronger immunosuppressive agents early in patients with endoscopic ulcers to avoid poor steroid response.

4.4 TNF-blocking antibodies

Since the expression of TNF in the gut is evidently elevated in patients with PD-1 inhibitor-induced colitis, it is theoretically feasible to target checkpoint-induced colitis with TNF blockade. Some scholars even laughingly describe anti-TNF as a magic bullet.

A retrospective study investigated the outcome of infliximab (IFX) therapy in eight patients of melanoma and lung and kidney cancers with severe steroid-resistant irAEs induced by ICIs. IFX was found to be effective in relieving colitis in these patients by neutralizing TNF-α, leading to the conclusion that early infusions of IFX in combination with systemic steroid therapy is necessary (56).

Etarnercept, known as a TNF-α cytokine-targeted antagonist, is widely used in the treatment of rheumatoid arthritis with outstanding efficacy and safety (57). When adding etarnercept to the colon cancer mice model with co-treatment of nivolumab and ipilimumab, Perez-Ruiz et al. noticed tumors remained well-controlled with no reduction in efficacy but ICIs-aggravated colitis was significantly alleviated (58).

The above experiments suggest the combined use of anti-TNF with ICIs may have a positive effect on efficacy and safety. It is reasonable to prophylactically neutralize TNF and appropriately increase the blood concentration of TNF antagonists to ensure low adverse reactions. We look forward to more real-world research conclusions to guide drug usage.

4.5 Vedolizumab

As a gut-selective α4β7 integrin antibody, vedolizumab (VDZ) inhibits T-cell migration to inflamed gastrointestinal tissues by blocking its binding to the gut mucosal addressin cell adhesion molecule 1 (MAdCAM-1). VDZ is approved for widely long-term use in inflammatory bowel diseases (59). After exploration by scholars, VDZ is also capable of effectively inhibiting ICIs-related corticosteroid-refractory gastrointestinal toxicity without hindering the antitumor effects (5).

In terms of the comparative efficacy of IFX and VDZ in immune-associated colitis, a multicenter study surrounding the role of VDZ therapy in ICI-induced steroid-refractory colitis was

conducted under the direction of Abu-Sbeih et al. After infusions of VDZ to patients who had been treated with steroids and/or IFX with poor results, approximately 86% of patients achieved clinical remission, 54% achieved endoscopic remission, and 29% achieved histologic remission (60). A two-center, retrospective study involving 184 patients (62 VDZ, 94 IFX, and 28 combination) clarified that there was similar oncological outcomes and clinical remission of colitis, but VDZ had a shorter duration of steroid therapy, fewer hospitalizations, lower recurrence rates of colitis than IFX, with the exception of a longer time to clinical response (61). As a consequence, VDZ is a potent alternative because of intestine-specific mechanism and efficacy for primary non-responders to anti–TNF-α.

Abu-Sbeih et al. pointed out that, analogous to the management protocol for IBD, selective immunosuppressive therapy (SIT) with IFX or VDZ monoclonal antibodies should be introduced early in the course of immune-mediated colitis (i.e., within 10 days of the onset), regardless of the response to corticosteroid therapy, as it allows for a shorter duration of clinical symptoms and fewer hospitalizations, while helping corticosteroid steroid dose reduction smoothly. SIT three and more infusions resulted in a better frequency of histological remission, lower fecal calprotectin levels, and more importantly, a distinct reduction in the recurrence rate of colitis (62). However, the heterogeneity of tumor types and dosing regimens of participants enrolled in the cohort makes this conclusion not equally applicable to all types of ICI-induced colitis, pending validation in prospective studies with larger samples.

It must be mentioned that, in spite of the positive feedback from patients on the combination of corticosteroids and biologics in many current studies, there is insufficient evidence on the risk and influence of OS in the immunocompromised advanced cancer population.

Several guidelines currently go into detail on these three weapons (corticosteroid, IFX, and VDZ), including the American Society for Clinical Oncology (ASCO), the European Society for Medical Oncology (ESMO), the National Comprehensive Cancer Network (NCCN), and a prior consensus statement from the Society for Immunotherapy of Cancer (SITC) (4, 63–65). Oral 1 mg/kg per day prednisone is recommended for G2 patients and G1 patients with positive lactoferrin as well as persistent or progressive diarrhea symptoms until symptoms improve significantly, followed by a taper over 4–6 weeks. Introduction of biologics within 2 weeks in addition to corticosteroids may be considered for those whose colitis is corticosteroids-refractory (i.e., no decrease by one grade in 3 days) or with high-risk endoscopic ulceration. IFX stands out as a preferred agent for those who do not respond to acute severe colitis, whereas VDZ is used as an alternative due to a mild delayed response. Three doses of IFX (5 mg/kg) are given at weeks 0, 2, and 6. If symptoms persist after the second dose of IFX administration, the third dose should be suspended and accordingly 3 doses of VDZ (300 mg) should be provided at weeks 0, 2, and 6. Repeat endoscopy to assess mucosal healing and fecal calprotectin surveillance may guide the timing of biologic dosing. Of note, to avoid reactivation of latent tuberculosis infection, VDZ is advisable rather than IFX for patients with tuberculosis, in which case the patient ought to obtain TB test before the first dose of biologics. Inpatient monitoring is strongly recommended for G3 and G4 patients. An intravenous prednisone or methylprednisolone should be administered at an initial dose of 1–2 mg/kg per day, followed by tapering over 4–6 weeks until symptom improve to G1 and switching from intravenous to oral at the appropriate time. If there is inadequate response after 3 days, IFX or VDZ may be added the same as G2. For patients with symptoms including fever, abdominal pain, and blood in the stool, abdominal CT scan should be performed immediately in order to rule out suspected complications and treat as soon as possible.

4.6 IL-1R antagonist

Current research indicates that targeted therapy with IL-1 receptor antagonists in autoimmune diseases such as Still's disease improves clinical outcomes (66). As mentioned above, the cytokine IL-1 performs an important part in the development of immune-related gastrointestinal adverse reactions. On the basis of this theory, Andrews et al. found with anakinra was sufficient to reverse dual ICIs-induced ileitis by blocking IL-1β without compromising the therapeutic efficacy in mice (16).

4.7 IL-6R inhibitor

Extensive attention has been drawn to clinical data that anti–IL-6 receptor monoclonal antibody (i.e., Tocilizumab and Sarilumab) is effective in high-level steroid-refractory colitis secondary to PD-1 blockers, while not compromising its efficacy. Thus, IL-6 antagonist may be a promising option for the steroid refractory irAEs with manageable safety profile.

After treatment with nivolumab, patients who developed severe colitis had a multiplicative increase in CRP from baseline. Given poor results with corticosteroids alone, Stroud et al. (14) added tocilizumab and noticed clinical remission followed by CRP decline to baseline or even lower. However, owing to the small sample size of this research and the lack of randomized trials to demonstrate the efficacy of Tocilizumab and its effect on PD-1 blocker therapy, further studies are desperately needed.

Similarly, Hailemichael et al. (37) retrospectively analyzed nearly 14,000 patients with melanoma, most of whom developed refractory irAEs after treatment with PD-1 blockers. With the addition of tocilizumab or Sarilumab to those who failed to improve after corticosteroids, the inflammatory indicators such as CRP and inflammatory cells were reduced; moreover, the significant increase in the assessment of patients' overall response rate to ICI revealed that IL-6 antagonists could enhance tumor immunity and attenuate the corresponding toxicity. It is a pity that there were no patients with gastrointestinal side-effects secondary to PD-1 therapy, so more experimental results are strongly expected.

Later Holmstroem et al. (67) revealed that Tocilizumab had a clinical benefit rate of up to 84% in ICI-induced colitis. By recruiting patients with CTCAE v5.0 grade > 1 ICI-induced colitis/diarrhea and adding Tocilizumab to non-stop ICI while

refusing hormones or immunosuppressants, they observed that the vast majority of patients achieved clinical remission (CTCAE reduction ≥ grade 1) within 8 weeks and hormone-free remission within 24 weeks.

However, contradictory to the above conclusion, preventive blockade of IL-6 in mice with tumors derived by subcutaneous transplantation of human MC38 colon cancer cells or B16-ovalbumin, anti-tumor efficacy of anti–PD-1 and anti–CTLA-4 combination in mice was partially reduced in case of Perez-Ruiz et al. (58).

Thus, we required further studies to confirm the role of IL-6 blockades in gastrointestinal toxicity and the optimal control dose.

4.8 Janus kinase inhibitor tofacitinib

The efficacy of Janus kinase (JAK) inhibitors stands out in the various inflammatory diseases, especially the potent JAK-selective inhibitor tofacitinib, currently found to serve as induction and maintenance therapy for ulcerative colitis (68). Due to the similarity of disease manifestations, its role in anti-PD-1–caused colitis has been explored.

A patient with gastric cancer developed colitis after pembrolizumab, presenting as refractory diarrhea, which was insensitive to successive glucocorticoids, infliximab and vedolizumab, and even if there was improvement, it recurred after dose reduction or even remained ineffective after increase in dose, while Esfahani and his colleague noticed that the diarrhea improved rapidly with the addition of third-line treatment with tofacitinib (69). Maybe tofacitinib act as a promising target with favorable outcomes and a good safety profile in refractory immune-related colitis.

4.9 Fecal microbiota transplantation

Gut microbes and their metabolites perform an unexpected function in cancer immunotherapy. We are looking forward to further breakthroughs with PD-1 inhibitors by reshaping microbes to enhance efficacy but minimize toxicity. The approach of fecal microbiota transplantation was then proposed and is proven by a growing number of scholars for its feasibility in dealing with PD-1 inhibitor-induced organ toxicity.

A patient with metastatic uroepithelial carcinoma developed grade 2 diarrhea/colitis after combination blockade of CTLA-4 and PD-1 and colonoscopy indicated severe colitis resembling ulcerative colitis. Another patient with prostate cancer received eprimar was hospitalized for fever and colitis and colonoscopy suggested Crohn's-like manifestations of ICI-associated colitis. After no relief with systemic corticosteroids, infliximab, and vedolizumab, they were treated with intestinal flora transplantation from a healthy donor, noticing rapid resolution of clinical symptoms and significant improvement in endoscopic evaluation, including regression of ulcers and reduction in inflammatory immune cell infiltration. Simultaneously, the type of bacteria with a protective effect was enriched after FMT treatment. The first patient was predominantly *Clostridia*, with a marked lack of *Bacteroidia* and *Verrucomicrobiae*. After FMT, there was a greater number of *Clostridia* and a significantly higher accumulation of *Akkermansia*, as well as an amplification of *Bifidobacterium*. The second patient was predominated by *Gammaproteobacteria*, such as Escherichia. After conducting FMT, the abundance of *Blautia* and *Bifidobacterium* increased noticeably. Puzzlingly, it was a gradual improvement of gastrointestinal symptoms, but the number of potentially pathogenic *Escherichia* first decreased and then increased significantly, while the beneficial *Bacteroides* increased and then eventually appeared to decrease. This case report delivered by Wang et al. highlights the role of FMT in rapidly and safely eliminating ICI-associated colitis after reconstitution of the gut microbiome (70).

Later, in a phase I clinical trial, fecal microorganisms from two patients, who had been previously treated with PD-1 inhibitors and achieved complete remission (CR) for more than 1 year, were transplanted to 10 patients who had failed anti–PD-1 therapy and had been depleted of intestinal flora by oral antibiotics. Then anti–PD-1 therapy was re-induced, and three of 10 persons showed clinical responses, including 1 CR. Analysis of their post-treatment tumor samples revealed upregulation of multiple immune-related genomes, such as IFN-γ–mediated signaling pathways, T-cell activation, and Th1 immune response. Although this study could not specify the flora profile that induces a clinical benefit for the time being, FMT and anti–PD-1 reprogrammed the TME and safely overcame immunotherapy resistance by modulating the intestinal flora (71).

FMT may become a first-line treatment when more evidence is evaluated. However, by means of FMT to overcome the anti-PD-1–caused toxic effects is not available to all institutions. Furthermore, there is insufficient evidence to guide the optimal parameters of FMT at present, such as donor requirements, operating technique, frequency, and applicable cancer types. Even though it is mechanistically possible to be an efficient therapeutic target, a variety of difficulties will make this novel therapy option challenging to spread.

4.10 The application of traditional Chinese medicine

The concept of "nourishing positive accumulation and eliminating cancer by itself" in traditional Chinese medicine (TCM) probably has the concept of eliminating tumors by restoring the body's immune system, which is identical to the tumor killing mechanism of PD-1 inhibitors (72). Increasing emphasis is being placed on TCM that act as immune checkpoint modulators (73). For instance, in combination with PD-1 inhibitors, ginsenoside Rg3 can function in diffuse large B-cell lymphoma and juzentaihoto (Shiquan Dabu Decoction) can function in B16 melanoma cancer (72). Mounting studies have demonstrated that TCM can modulate the TME and related effectors to influence clinical outcomes.

Baicalin, a bioactive component of Scutellaria baicalensis, is capable of reducing the levels of inflammatory factors IL-1β, TNF-α, and IL-6, as well as blocking the PD-1 signaling pathway by inhibiting IFN-γ, thereby playing an essential role in anti-inflammation and anti-tumor efficacy of PD-1 antibodies (74).. Of

note, baicalin promotes an increase of intestinal bacteria producing SCFA (especially butyrate), which may theoretically inhibit the occurrence of irAEs (74).

Meng et al. (75) found that PD-1 inhibitors in melanoma mice may lead to an increase in Th2 cells and secretion of IL-4, IL-5, and IL-10, which have a negative effect on antitumor effects. The addition of Brucea javanica oil emulsion (BJOE) to anti–PD-1 revealed not only successfully reversed the suppressed TME with reduced Tregs levels but also showed significant synergistic anti-tumor effects with sound biosafety, as manifested by a notable inhibition of tumor growth, improved survival rate in mice without weight loss, and an increase in $CD4^+$ T cells, $CD8^+$ T cells and the proportion of MI/M2 macrophages as well as an increase in TNF-α, IFN-γ, CXCL10, and granzyme B.

When Gegen Qinlian decoction, constituted from *Radix Puerariae*, *Scutellariae Radix*, *Coptidis Rhizoma*, and *liquorice* in certain proportions, was combined with anti-mouse PD-1 in mouse models of colorectal cancer, Lv et al. noticed that tumor growth was significantly inhibited, intestinal flora *Bacteroidales_S24-7_group* was remarkably abundant, as well as increased levels of $CD8^+$ T cells and IFN-γ in tumor tissues and decreased levels of PD-1 (76). This combination therapy enhances anti-colorectal cancer activity when it fails to respond to anti–PD-1 agents, by reshaping the intestinal flora and restoring T-cell function.

Overall, since the mechanism of herbs enhance anti-tumor activity without adverse effects as mentioned above, perhaps its combination therapy with anti–PD-1 can be popularized in more cancer patients.

4.11 Artificial intelligence

AI is penetrating into our daily life, including a growing role in cancer immunotherapy. Scholars found to their surprise that AI can be prospectively used for immunotherapy response prediction. Predictive models are built by AI combining with big data, including radiomics, genomics, proteomics, transcriptomics, pathology imaging, and clinical characteristics (77). This AI-based clinical decision-making system promotes circumventing differences caused by heterogeneity of tumor immune microenvironment and identifying patients who benefit most from ICI with minimal adverse clinical effects, so as to help clinicians develop individualized treatment plans (78).

Although AI has yet to mature further and require large public database of different centers to improve accuracy, we can be confident that, in the future, it can help build powerful predictive models and shine in the field of precision oncology (Table 2).

5 Predictive biomarkers

Scholars are devoted to investigate biomarkers that can predict gastrointestinal toxicity early, which are mentioned in Table 3. Nevertheless, tumor heterogeneity may result in restrictions on the application of biomarkers. A greater number of prospective studies are required to put them into clinical practice.

TABLE 2 Multiple treatment strategies for addressing irAEs.

Therapeutic methods	Specific details
Pre-treatment preparation	Detection of intestinal inflammatory markers like fecal lactoferrin and calprotectin; Screening for Clostridium difficile and CMV infections in feces; Determination of celiac disease by tTG-IgA; Early use of flexible sigmoidoscopy or colonoscopy with biopsy (53).
Symptomatic treatment	A low-fiber dietary; Antidiarrheal drugs like loperamide; Add mesalamine or cholestyramine if necessary.
Corticosteroid	Individualized hormone application; Use the lowest effective dose of corticosteroids for the shortest period of time (54).
TNF-blocking antibodies	Combined use of ICIs with anti-TNF agents such as infliximab or etarnercept (56, 58).
Vedolizumab	Vedolizumab therapy in ICI-induced steroid-refractory colitis or non-responders to anti–TNF-α (60, 61); Early introduction (within 10 days of the onset) of VDZ and sustained 3 or more infusions (62). Available for tuberculosis patients.
IL-1R antagonist	Reversal of dual ICIs-induced ileitis with anakinra (16).
IL-6R inhibitor	Use of Tocilizumab or Sarilumab for refractory irAEs (14, 37, 67).
Janus kinase (JAK) inhibitor	Application of the potent JAK-selective inhibitor tofacitinib in refractory immune-associated colitis (69).
Fecal microbiota transplantation (FMT)	Remodeling of the gut microbiota by receiving intestinal flora from a healthy donor (70, 71).
Traditional Chinese Medicine	Joint application anti–PD-1 drugs with Baicalin (74), Brucea javanica oil emulsion (BJOE) (75) and Gegen Qinlian decoction (76).
Artificial intelligence (AI)	As an assistive technology, AI can be combined with big data to build an AI-based clinical decision-making system for minimal adverse reactions (77, 78).

TABLE 3 Exploration of biomarkers for early prediction of irAEs.

Biomarkers	High risk of irAE occurrence	Low risk of irAE occurrence
Blood counts and biochemical indicators	Lower NLR (< 3) and PLR (< 180) as well as higher AEC (> 240/μl) at baseline (79); Performance status (PS) ≥ 2 and albumin levels < 35 g/liter (80).	
Genetic susceptibility	The presence of HLA-DQB1*03:01 (81); Higher expression levels of LCP1 and ADPGK (17); Mutations in the NRAS gene (p = 0.2844) (82).	Mutations in the oncogenic gene BRAF (p = 0.0180) (82); With the SNPs (CYP24A1 rs2762934, CYP27B1 rs10877012, and SEC23A rs8018720 genotypes) (83).
Dynamic circulating tumor DNA (ctDNA)	With alterations in the CEBPA, FGFR4, MET, or KMT2B genes in ctDNA (84).	
C-reaction protein (CRP)	Elevated CRP levels that CRP ≥ 8.2 mg/liter (P = 0.024) (85).	
Extracellular vesicle-derived proteins	Low levels of EV-ICOS and EV-IDO1 (86).	Highly expressed EV-ICOS and EV-IDO1 (86).

5.1 Blood counts and biochemical indicators

Circulating blood counts, such as neutrophil-lymphocyte ratio (NLR), platelet lymphocyte ratio (PLR), absolute eosinophilia count (AEC), and absolute basophil count (ABC) often objectively foresee the onset time of irAEs. Lower NLR (< 3) and PLR (< 180) were noticed in the irAE group, whereas higher AEC (> 240/μl) at baseline were related with endocrine irAEs (79).

A prospective follow-up of irAEs involving 1,187 cancer patients using anti–PD-1/L1 surprisingly identified factors associated with poor prognosis in solid cancer patients, such as elevated NLR and performance status (PS) ≥ 2 predicted very severe irAE and fatal irAE, and albumin levels < 35 g/liter predicted fatal irAE (80). Although the highest mortality rates have been reported for respiratory, cardiovascular, and nephrotoxicity, rather than gastrointestinal toxicity, it is worthwhile to alert clinicians to make early decisions so as to avoid serious gastrointestinal adverse events that affect patient quality of life and prognosis.

5.2 Genetic susceptibility

Genetic background of patients receiving ICI probably functions in irAE susceptibility.

It is clarified that the human leukocyte antigen (HLA) complex located at chromosome 6p21.3 is highly associated with the development of IBD, for example, HLA-DRB1*03:01 is the most relevant risk allele (87). However, it is unclear whether genetic HLA-mediated susceptibility to autoimmune disease contributes to the development of irAEs. Surprisingly, after analyzing the HLA alleles of nine patients who developed colitis after ICI treatment, Ali et al. found a nominally significant association between HLA-DQB1*03:01 and colitis, in other words, carriers of which were susceptible to colitis during immunotherapy (81). Owing to the very limited sample size, no insight into HLA-associated disease dynamics is possible, however, and it is essential to recruit a larger cohort for a more extensive genetic investigation.

The lymphocyte cytosolic protein 1 (LCP1) is involved in T-cell activation and the adenosine diphosphate dependent glucokinase (ADPGK) mediates metabolic shifts during T-cell activation. Combining real-world data with multi-omics data, Jing and coworkers developed a bivariate regression model for LCP1 and ADPGK, and validated it in a cohort of cancer patients receiving anti–PD-1/PD-L1 inhibitors, revealing the highest correlation between these two values and the presence of irAEs.

LCP1 (P = 0.008) and ADPGK (P = 0.010) were higher in patients with irAEs than in those without irAEs, which suggests LCP1 and ADPGK may serve as biomarkers in an independent patient-level cohort (17).

Another retrospective study discovered that mutations in the oncogenic gene BRAF induced a lower probability of irAEs (p = 0.0180), while mutations in the NRAS gene may be positively associated with the development of irAEs (p = 0.2844). However, given the lack of prospectively collected mutational status, the association of oncogenic drivers with irAE occurrence requires further exploration to unravel (82).

A growing body of research has recently demonstrated the benefits of Vitamin D in tumor management by inhibiting tumor angiogenesis and microenvironmental inflammation (88). With Luo et al. (83) genotyping of 13 single-nucleotide polymorphisms (SNPs) in the Vitamin D metabolic pathway demonstrated that patients with the *CYP24A1* rs6068816TT and rs2296241AA genotypes were more likely to benefit from anti–PD-1 drugs. Three SNPs (*CYP24A1* rs2762934, *CYP27B1* rs10877012, and *SEC23A* rs8018720) reduced the risk of irAE in patients. However, although plasma 25(OH)D levels were associated with good disease control rates in patients, there was no statistical correlation with the risk of irAE.

5.3 Dynamic circulating tumor DNA

With assessment of 46 patients with advanced gastric cancer (AGC) who received anti–PD-1 immunotherapy and next-generation sequencing (NGS) testing, Jin and his colleagues

observed the median PFS of 7.4 months *versus* 4.9 months for patients with undetectable and detectable post-treatment ctDNA (84). In other words, lower ctDNA was associated with a higher response to immunotherapy.

Common adverse reactions in enrolled patients were endocrine manifestations and hepatotoxicity, which were more likely to occur in patients with alterations in the CEBPA, FGFR4, MET, or KMT2B genes in ctDNA (84). So dynamic ctDNA is hopeful to be a biomarker for predicting irAEs, and we expect a larger cohort to conduct further exploration of its potential role in gastrointestinal toxicity.

5.4 C-reaction protein

C-reaction protein (CRP) is an acute phase protein synthesized by the liver and is markedly elevated in the early stages of inflammation or infection. Baseline CRP values cannot reliably predict irAE, but elevated CRP levels seem to correlate with irAE, with researchers noting that CRP ≥ 8.2 mg/liter (P = 0.024) were independent risk factors (85). However, because CRP is inherently elevated in patients with malignancy and the use of hormones can have an impact on its measurement, it is necessary to pay attention to whether the reduction in CRP correlates with improvements in other factors when assessing the recovery of patients with irAEs.

5.5 Extracellular vesicle-derived proteins

Extracellular vesicles (EVs) are membrane-bound nanovesicles that are secreted and released by almost all cells carrying a variety of bioactive molecules such as DNA, mRNA and proteins. Not only are they involved in signal transduction in intercellular settings, but a growing body of evidence clarifies that they are also likely to be non-invasive markers for cancer diagnosis and treatment (89). According to recent evidence, inducible co-stimulator (ICOS), served as a co-stimulatory receptor for T-cell enhancement, plays a dual role in different tumor types. As it enhances the production of CD8+ tissue-resident memory (Trm) T cells to strengthen the anti-tumor response, while promoting tumor progression by depressing the function of Tregs (90, 91). Indoleamine 2, 3-dioxygenase 1 (EV-IDO1) is a rate-limiting metabolic enzyme highly expressed in a variety of human cancers, although its functional effects in different cancer types are complex and remain to be investigated in relation to prognosis (92). Increased expression of IDO1 in tumors is accompanied by an increase in other immune checkpoints such as PD-1 (92). Hence, treating PD-1 inhibitors together with IDO1-targeted blockers may have a synergistic effect on immunotherapy, but whether there is a corresponding risk of additional toxicity is still unknown.

EV-ICOS and EV-IDO1 were extracted from the peripheral blood of patients with gastric cancer. Jiang et al. (86) reported firstly that these two EV-proteins were highly expressed in patients without irAEs. In addition, for the time interval between the initiation of ICI therapy and the development of irAEs, patients with high-baseline ICOS and IDO1 were slightly longer than those with low baseline ICOS and IDO1 expression. That is, patients carrying low levels of EV-ICOS or EV-IDO1 had a higher risk of irAEs and a shorter interval. Interestingly, these two proteins neither affected the long-term immunotherapy outcome that no changes in PFS and OS were seen, but the levels of the circulating tumor marker CA72.4 were highly positively correlated with them, meaning that they may affect short-term efficacy. Furthermore, EV-derived ICOS/IDO1 was not sufficient to predict organ-specific irAE.

6 Risk factors: individualized toxicity monitoring

Regarding the correlation between age and irAEs, scholars have conducted studies. Treating tumor-bearing mice with PD-1 inhibitors, Tsukamoto et al. noted significant aberration in biochemical indicators of organ dysfunction in aged mice, such as elevated alanine aminotransferase (ALT), triglyceride, amylase, urea nitrogen, ureic acid, surfactant protein D (SP-D), and abnormal lymphocytic accumulation, while juvenile mice unexpectedly showed no obvious irAEs-Like multi-organ toxicity (32). To study the response of elderly patients to treatment with PD-1 inhibitors, the group of 82 patients aged 65–79 years was defined as group A and the group of 62 patients aged 80–100 years was defined as group B. The investigators noted a trend toward higher ORR in group B compared with group A (73.9% *vs.* 62.3%) and a significantly higher CR rate in group B than in group A (47.9% *vs.* 20%), meaning that older melanoma patients over 80 years of age benefited the most. A weakened immune system with age may result in a better response to anti–PD-1 therapy. There was no significant difference in PFS or OS between the groups, probably because a considerable number of elderly patients were prone to die from complications other than melanoma progression such as ischemic heart disease. However, toxicity occurred significantly earlier in group B, that is, patients over 80 years of age, and may be related to more rapid kinetics of immune activation (93). Therefore, aging accelerates the disruption of immune tolerance, so that give rise to earlier onset of irAEs.

About the impact of gender, a prospective study of ipilimumab for melanoma clarified that women are at a higher risk of serious toxicity relative to men (94). In contrast, men who used PD-1 drugs and long-term smokers were at higher risk (79). Contradictory to findings mentioned above, in patients with cancer undergoing PD-1 blockade, neither age nor gender was noticed to influence the development of irAE, but high BMI (BMI > 25.0) made greater odds of irAE (82). Moreover, patients with underlying diseases such as hypertension, coronary heart disease, and chronic kidney disease have an elevated organ-specific irAE risk (79). Primary tumor histology also has an influence that distinct tumor types have histologically specific irAE patterns when treated with PD-1 suppressors, perhaps attributed to diverse TMEs and immune infiltration (81), with research identifying a higher risk of small bowel colitis in melanoma compared with NSCLC and renal cell carcinoma (95).

That irAEs attribute to dysregulation of immune system is resemble autoimmune diseases like IBD (23). Several studies have indicated that preexisting autoimmune disease increases the risk of irAE, probably because of enrichment of activated $CD4^+$ T_M cells (15, 79). Not only that, Kayashima et al. noticed irAEs could lead to the follow-up progression of IBD (96). An elderly male with a previous history of left-sided involvement in ulcerative colitis was treated with anti–PD-1 drugs after the appearance of multiple organ metastases from kidney cancer, and subsequently experienced immune-mediated colitis with a primary site in the right transverse colon. Upon administration of prednisolone and multiple IFX to the patient, the condition was poorly controlled or even newly worsened, with inflammation extending to the rectum and sigmoid colon, which implies a realization of a shift in pathogenesis from irAE to ulcerative colitis recurrence (96). Whether to treat such patients with ICIs remains controversial, out of concern for symptom deterioration. In any case, frequent colonoscopies may be valuable for the management of such patients.

Early identification of high-risk patients facilitates diagnosis and timely treatment, although comprehensive predictive models are currently inaccessible (54).

7 The relationship between efficacy and safety

Does the development of irAEs affect the antitumor immune effect of ICI? How exactly does irAEs correlate with the survival outcomes and prognosis of anticancer process? In a large retrospective study investigating patients with various cancers treated with anti–PD-1 drugs, there were clinical benefits identified as remarkably higher objective response rate (ORR) (30.4% *vs.* 12.7%), notably prolonged median progression-free survival (PFS) (17.6 months *vs.* 3.0 months) and overall survival (OS) (48.7 months *vs.* 10.7 months) in patients with irAEs than in those without irAEs (97). Another meta-analysis including 4971 individuals showed irAEs, particularly low-grade irAEs, can be acted as predictive factors of a better ICI efficacy and prognosis, regardless of disease site (98). Relatively speaking, irAEs above grade 3 resulted in better ORR, but worse OS (99). Giving nivolumab to patients with AGC, Masuda et al. revealed the absence of irAEs indicated poor prognosis by multivariate analysis (100). Kono et al. also endorsed this view, contending a strongly positive association between irAEs and clinical benefit such as a longer OS in nivolumab-treated patients with AGC (101). Likewise, Jiang et al. noticed the occurrence of irAEs corresponds to a better disease control rate of immunotherapy in gastric cancer patients (86). Even, patients with multiple irAEs had a trend toward improved PFS and OS, as compared with patients with only a single irAE (82). Speaking from the studies listed above, irAEs mirror an early and timely immune activation against the tumor that should be considered as a surrogate marker for a positive response to ICI therapy (102). It is uncertain whether interrupted ICI therapy will limit long-term benefit on survival (103).

8 Rechallenge after irAEs

Scholars increasingly recognize that both the antitumor activity of ICIs and the accompanying adverse effects can be attributed to an overreaction of the immune system. There are a lot of debates if immunotherapy can be restarted after the onset of irAE.

In a large multicenter retrospective study, for the purpose of assessing the safety of ICI resumption after irAE, the researchers focused on patients who discontinued ICI therapy for episodes of immune-mediated diarrhea and colitis and resumed ICI therapy due to cancer progression or favorable response for continued maintenance therapy, it was detected that up to one-third of patients suffered colitis recurrence and must permanently cease ICI (104). But patients who used anti–PD-1/L1 drugs before the initial colitis episode had a higher risk of recurrent colitis; those who used anti–CTLA-4 at the time of ICI readministration had a higher risk of recurrence than those who used anti–PD-1/L1 and had a shorter time interval, which may be attributed to anti–CTLA-4 induced stronger Th17 memory in colitis than anti-PD/1, although no difference in severity was noted by Abu-Sbeih et al. (37, 104). Interestingly, compared with pembrolizumab, nivolumab appears to have a higher mean incidence of grade 3 or higher adverse events with an unknown mechanism (9).

Patients were closely monitored and it was seemingly safe to restart ICI therapy, as demonstrated by the fact that 61.1% of patients who discontinued ICI therapy owing to grade ≥ 2 irAEs did not experienced a recurrent grade ≥ 2 irAEs after reintroduction of the same ICI therapy (105). By analyzing case safety reports from the World Health Organization database VigiBase, Dolladille, and fellow revealed a 28.8% recurrent rate of the same irAE in patients retreated with the same ICI after an irAE. In a readministration, colitis was associated with a higher recurrence rate compared with other events (106). Despite the size, this cohort study did not assess differences in the severity of the two irAEs. According to Simonaggio et al., a second identical or different irAE of patients who were rechallenged with the same anti–PD-1/L1 agent were in a similar degree of severity to the first, but the interval between recurrent events was significantly shorter than the initial one (107). Concerning the observation of curative effect, the ORR and DCR after rechallenge were 43.1% and 71.9%, respectively, with no significant difference in comparison with the initial ICI treatment (108).

The guidelines have the following recommendations. Patients with G1 irAE generally continue ICI therapy but are monitored closely to avoid any symptom deterioration. G2 irAE patients should temporarily discontinue treatment and subsequently restart if there is a hormone reduction (e.g., prednisone ≤ 10 mg/day) and symptoms return to G1 or even disappear completely. Provided that the subjective desire of G3 irAE patients is fully considered, clinicians should evaluate whether the patient will gain benefit from resumed therapy without undue deliberation about the presumed risk of toxicity to influence decision making. To minimize the risk of recurrence of irAEs, the ASCO Guideline suggests that mucosal healing in repeated

endoscopies and/or fecal calprotectin levels ≤ 116μg/g can be considered as indicators of the time to resume ICI therapy (64). Moreover, anti–PD-1/L1 drug therapy should be the preferred option, regardless of the initial regimen. G4 patients generally recommends permanent discontinuation of ICIs. However, patients with a history of autoimmune disease were not included in any of these studies, and potentially increased risk was not taken into account. In general, the decision to rechallenge is actually based on the potential risk-reward ratio, further large-scale prospective studies are unquestionably required to more thoroughly validate.

9 Rare cases

In the past few years, the rare gastrointestinal toxicity caused by PD-1 inhibitors has gained attention. Despite its rarity, it should be emphasized because delays in treatment may lead to a poor prognosis or even death.

9.1 Upper gastrointestinal inflammation

Mild upper gastrointestinal inflammation can be effectively managed with proton pump inhibitors, but deep ulcers usually require immunosuppressive therapy. After anti–PD-1 therapy, Collins et al. observed that some patients suffering from immune-associated colitis developed upper gastrointestinal inflammation in the form of $CD8^+$ lymphocyte infiltration, manifested by dysphagia, nausea and vomiting, and even progression to necrotic gastritis. Up to 75% of them responded favorably to corticosteroids, so the application of corticosteroids seems necessary and effective in the case of severe toxicity (109).

9.2 Bowel perforation

Cho et al. presented a case of an esophageal cancer patient who underwent a second dose of nivolumab with complaints of abdominal pain and diarrhea. After the ineffectiveness of corticosteroids and antibiotics, a diagnostic laparoscopy revealed a perforated cecum with severe abdominal wall adhesions, severe inflammation and multiple ulcerations in the sigmoid colon, so that the patient was given an ileostomy ultimately until recovery from colitis (110).

A case is reported by Celli et al. of a melanoma patient treated with pembrolizumab who developed diffuse colitis that responded poorly to high-dose corticosteroids and IFX and even progressed rapidly to a fulminant colitis with multifocal ulcers and perforation, culminating in an emergent bowel resection (111).

In general, continuation of ICI therapy is not taken into consideration after the development of severe complications. However, Beck et al. reported a case of exceptional management. After pembrolizumab, a patient with lung cancer suffered from immune-mediated enterocolitis that progressed to small bowel perforation and ended up with partial small bowel resection and creation of a primary anastomosis. Considering that pembrolizumab was tolerated fairly well, tumor burden was significantly attenuated, and satisfactory outcome to treatment, it was still decided to reactivate pembrolizumab therapy (95). Whether permanent discontinuation of anti–PD-1 drugs after perforation is necessary needs to be decided carefully after taking into account the individualized therapy situation.

9.3 Intestinal obstruction

A case of colitis and secondary inflammatory intestinal obstruction in a liver cancer patient with sintilimab was recently reported. Tan and coworkers pointed out that surgery, a traditional means of relieving obstruction, should not be undertaken in order to avoid aggravating intestinal damage and postoperative complications (112). Furthermore, gastrointestinal decompression and parenteral nutrition in combination with glucocorticoids and somatostatin, a type of hormone known to inhibit the secretion of digestive juices and suppress inflammation, should be administered early to patients with severe abdominal distention and decreased anal exhaust after diagnosis of colitis to accelerate remission (112).

9.4 Celiac disease

Celiac disease (CeD) is a relatively rare form of irAE whose pathogenesis may be related to gluten-mediated activation of intestinal $CD4^+$ T cells in the lamina propria (113). It is clinically identical to both duodenitis and colitis, with the exception that it usually does not require immunosuppressive therapy, so that early diagnosis by the presence of anti-tissue transglutaminase immunoglobulin (tTG-IgA) is crucial (114).

A recent case of an elderly male patient with melanoma receiving combination nivolumab and ipilimumab, presented with abdominal pain, diarrhea and generalized edema, positive serology for tTG-IgA, endoscopy suggestive of duodenal mucosal atrophy with duodenal biopsies confirming fulminant celiac disease. This patient improved with a gluten-free diet alone, defined as a decreasing trend in tTG-IgA, rather than immunosuppression (115).

Lacking appropriate biomarkers, clinicians warrant high vigilance for patients presenting with gastrointestinal distress after ICI therapy, preferably with early access to tTG-IgA titers. In case of non-response after empirical use of corticosteroids, a full gastrointestinal endoscopy with biopsy should be performed to clarify the etiology and prevent the emergence of fulminant events.

9.5 Appendicitis

The clinical manifestations (e.g., abdominal pain and fever) and imaging manifestations (e.g., appendiceal dilatation and wall thickening) of appendicitis after PD-1/PD-L1 inhibitors are

similar to those of conventional appendicitis, so the current management strategies are largely similar (116). Despite the complexity of cancer patients, appendectomy remains the mainstay of treatment. Distinction between ICI-mediated colitis and appendicitis is of concern and is critical for follow-up. Diarrhea often occurs in cases of colitis. Moreover, a considerable proportion of colitis events will be early treated with hormones in the course of the disease, while antibiotics are not used because of negative effect on long-term survival (117); whereas in this case, appendicitis was managed without hormones or immunosuppression, instead, antibiotics were often used. Additionally, microbiota analysis has led to an improved understanding of colitis, and FMT has been shown to be effective in ICI-mediated colitis. Perhaps future studies with larger sample sizes can reveal that the microbiome characteristics of appendicitis patients to allow FMT to be equally beneficial for appendicitis patients. Improving the understanding of this adverse event is a critical unmet need to determine whether to continue ICI treatment after an appendicitis episode.

10 Conclusion

With the widespread application of PD-1 inhibitors, it brings satisfactory efficacy but also affects the patients' life quality due to unpredictable irAEs. How targeted eliminate the adverse effects without compromising the antitumor activity of immunotherapy has become a continuously explored topic by scholars. We expect that a more comprehensive summary of the gastrointestinal adverse effects caused by PD-1 will lay the theoretical foundation for the improvement of relevant management measures in the future.

Author contributions

YYC drafted the manuscript and generated the figure. FL and JL performed the background research. YDC, MX and SL were responsible for the documentation. LZ conceived of and designed the study as well as revised and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Immune-related adverse events associated with nab-paclitaxel/paclitaxel combined with immune checkpoint inhibitors: a systematic review and network meta-analysis

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Objective: The combination of nanoparticle albumin-bound paclitaxel (nab-PTX)/paclitaxel (PTX) with immune checkpoint inhibitors (ICIs) has demonstrated significant efficacy in cancer patients. However, the safety of these combination regimens remains conflicting in former researches. Therefore, in order to address this issue, we performed a systematic review and network meta-analysis (NMA) to evaluate and compare the safety profile.

Methods: We performed a systematic review by searching randomized controlled trials (RCTs) from PubMed, EMBASE, Cochrane Library, ClinicalTrials.gov, and Web of Science up to August 15, 2022. The primary outcomes were all-grade (grade 1-5) and high-grade (grade 3-5) immune-related adverse events (irAEs). Secondary outcomes were all-grade (grade 1-5) and high-grade (grade 3-5) irAEs of subgroups of ICIs.

Results: There were 22 RCTs included in the NMA, involving a total of 15 963 patients diagnosed with any type of cancer. ICIs+nab-PTX was associated with a noticeably decreased risk of grade 3-5 pneumonitis (odds ratio [OR]=0.28, 95% credible interval [CrI]: 0.09,0.90) compared to ICI monotherapy; ICIs+PTX showed a lower risk of grade 1-5 hyperthyroidism (OR=0.46, 95% CrI: 0.22-0.96) and grade 1-5 hypothyroidism (OR=0.49, 95% CrI: 0.26-0.93) than ICIs. Compared with PD-1, PD-1+PTX was associated with a statistically significantly lower risk of grade 1-5 pneumonitis (OR=0.32, 95% CrI: 0.11-0.92). PD-L1 resulted in a noticeably lower risk of grade 1-5 hypothyroidism (OR=0.34, 95% CrI: 0.12-1.00) than PD-L1+PTX. Nearly all treatment regimens containing ICIs demonstrated significantly higher risks of irAEs compared to the standard chemotherapy groups.

Conclusion: Nab-PTX/PTX+ICIs demonstrated an approach leading to decreased risk of irAEs compared with ICI monotherapy. This finding supports that ICIs+nab-PTX/PTX may be a safer treatment strategy. Moreover, we also found that the combination regimens containing ICIs had a higher risk of irAEs than standard chemotherapy. Additionally, ICIs+nab-PTX demonstrated a decreased risk of irAEs compared to ICIs+PTX. PD-1 inhibitors were associated with a higher risk of irAEs than PD-L1 inhibitors.

KEYWORDS

immune-related adverse events, systematic literature review, network meta-analysis, immune checkpoint inhibitors, nab-paclitaxel, paclitaxel

1 Introduction

Immune checkpoint inhibitors (ICIs) targeting programmed cell death 1 (PD-1), programmed cell death ligand 1 (PD-L1), or cytotoxic T lymphocyte antigen 4 (CTLA4), has been become one of the most important breakthroughs in cancer therapy (1). Immune suppression plays a key role in cancer growth and progression. ICIs promote immune responses against tumor cells by blocking immune checkpoint pathways. Treatment targeting immune checkpoints, such as anti-PD-1, anti-PD-L1, and anti-CTLA4 demonstrate impressive anti-tumor activities against several tumor types (2). Over the past decades, monoclonal antibodies against the PD-1/PD-L1 pathway have been approved for melanoma, prostate cancer, lung cancer, liver cancer, cervical cancer, gastric cancer, and breast cancer (3). However, a large proportion of patients do not respond or even resistant to ICIs (4–6). In several clinical trials (7–9) using biomarkers to predict the treatment response to anti–PD-1 or anti–PD-L1 therapies, the objective response rates were still unsatisfied (<50%).

Current research had been focused heavily on the improvement of the response rate of ICIs. Taxane-based chemotherapies, including albumin-bound paclitaxel (nab-PTX) and paclitaxel (PTX) (10–13), might have a "priming effect" for the immune system and improve the response to the ICIs (14). Although the "priming effect" of chemotherapy is still unexplained, ICIs combined with nab-PTX/PTX demonstrated superior efficacy in multiple clinical trials (13, 15). ICIs combined with nab-PTX/PTX has been widely adopted in the clinical practice (16), even though the combination therapy is not strongly recommended by the National Comprehensive Cancer Network (NCCN) (17–19).

Despite the substantial clinical benefits associated with ICIs +nab-PTX/PTX (20, 21), there has been rising concerns on the safety of combination therapy. ICIs may result in the activation of the immune system and are associated with adverse events, which are known as immune-related adverse events (irAEs) (22) (23, 24). The irAEs (22) include rash, colitis, hepatitis, hypothyroidism, hyperthyroidism and pneumonitis, occurring in up to 70% of patients treated with ICIs. The irAEs (22) could be severe or even fatal (22, 25), but their mechanism is still unclear. The NCCN has released several guidelines addressing adverse events associated with ICIs. For the combination of ICIs and nab-PTX/PTX, the synergistic effect of the combination strategy may attribute to therapy-associated cytokine release and T-cell-mediated organ infiltration (10, 11, 13, 26–30), leading to the alterations in safety profiles. However, the safety profiles of ICIs and nab-PTX/PTX is still inconsistent in the literature. Previous studies suggested that ICI alone is generally better tolerated than combination regimens (26, 31), but more recent studies concluded that ICIs and nab-PTX/PTX combination regimen demonstrated better safety profiles (32, 33). To our best knowledge, there are limited studies investigating the safety of ICIs+nab-PTX/PTX. Past meta-analyses mainly focused on a specific ICI or nab-PTX/PTX, failing to cover possible combination therapies (26, 34–36). With more ICIs on the market, it is very important to compare the safety profiles between combination regimens, but the head-to-head comparison is largely lacking. Therefore, we conducted a network meta-analysis (NMA) to comprehensively evaluate the safety profile and safety ranking of nab-PTX+ICI, PTX+ICI, ICI monotherapy and chemotherapy. This approach allowed us to combine direct and indirect evidence and rank the interventions based on their relative safety profiles.

2 Methods

This study was registered in the Prospective Register of Systematic Reviews (CRD42022326742). This NMA followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) and the PRISMA extension statement for network meta-analysis (37).

2.1 Data sources and searches

We conducted a comprehensive search of relevant studies using keywords in electronic databases, including PubMed, Embase, Cochrane Library, Web of Science and ClinicalTrials.gov, between January 1, 2000 and August 15, 2022. Search key terms used in the

search strategy include cancer, oncology, nab-PTX/PTX, immune checkpoint inhibitors, randomized controlled trials. The search strategy is described in the Supplementary Table 1. Two reviewers (WJ and JZ) firstly screened the titles and abstracts, then reviewed the full-texts of publications. Any discrepancies were resolved through discussion and consultation with the third reviewer (YW).

2.2 Study selection criteria

The study had pre-defined inclusion and exclusion criteria. Inclusion criteria were: (1) phase II or phase III randomized clinical trials (RCTs) with head-to-head comparison; (2) trials typically involve at least two arms the following mentioned: one ICI drug (PD-1/PD-L1 inhibitors), one ICI drug in combination with nab-PTX/PTX, one ICI drug in combination with chemotherapy; (3) study subjects diagnosed with cancer, (3) reported the incidence and grade of adverse events; (4) written in English.

The publications were excluded with any of the following: (1) letters, abstracts, reviews, posters, conference reports, unfinished studies or duplicated reports; (2) trials with insufficient data; (3) single-arm studies; (4) phase I randomized trials; (5) cost-effectiveness studies.

2.3 Data extraction

Two reviewers (WJ and JZ) extracted data independently, including first author, year of publication, treatment line, type of ICIs, stage of the cancer trial phase, treatment arm, incidence of grade 1-5 and grade 3-5 irAEs, sample size, patient age, sex distribution, cancer type, PD-L1 expression, Performance Status (PS) score, median follow-up time and Common Terminology Criteria for Adverse Events (CTCAE) edition.

In terms of adverse events data, because immune-related adverse events were the outcome of interest, we first evaluated "immune-related adverse events" in the main text and Supplementary Materials of published studies. We also screened all possible information available at ClinicalTrials.gov to obtain a more comprehensive data extraction. If irAEs were not available in the study (n=2), treatment-related adverse events were used and extracted.

2.4 Quality assessment

We used the Cochrane Collaboration's risk of bias tool (38) to assess the quality of each trial included. Two reviewers (WH and YW) assessed on the 5 aspects, including the random sequence generation, allocation concealment, blinding, outcomes assessment, and reporting. Each aspect was graded based on the risk of bias, categorized by yes, no, or unclear. Any discrepancies in data extraction and quality assessment disagreements were resolved by discussion to achieve a consensus.

2.5 Statistical analysis

We summarized characteristics of trials, including first author, year of publication, treatment line, type of ICIs, stage of the cancer, trial phase, treatment arm, incidence of grade1-5 and grade 3-5 irAEs, sample size, patient age, sex distribution, cancer type, PD-L1 expression, PS score, median follow-up time and CTCAE edition. We accessed on the total number of all irAEs and the number of each specific irAEs, respectively. Incidence rates of grade 1-5 and grade 3-5 irAEs were compared among different treatment regimens, including chemotherapy, ICI monotherapy, ICI+nab-PTX and ICI+PTX. To investigate whether the occurrence of irAEs was influenced by the type of ICIs (PD-1 and PD-L1), we further subdivided the four treatment groups into six subgroups based on different types of ICIs: chemotherapy, PD-1 monotherapy, PD-L1 monotherapy, PD-1+PTX, PD-L1+PTX and ICIs+nab-PTX. Considering the relatively small sample sizes of PD-1+nab-PTX and PD-L1+nab-PTX, they were combined into one group.

To evaluate the statistical heterogeneity of the included trials, we accessed the I^2 index and the Cochran Q statistic. Heterogeneity was defined as low for I^2 values as 25–49%, moderate for 50–74%, and high for >75%, respectively. For NMA, we generated network plots depicting direct and indirect comparisons using STATA V.17.0. We used ADDIS-1.16.6 for head-to-head direct meta-analyses. To evaluate the risk of irAEs, we calculated ORs and 95% CIs using the random effects model, to account for unexplained heterogeneity. The random effects model is considered to be the most conservative method (39). Two-sided $P<0.05$ was considered significant.

Due to the potential low irAEs rate and limited sample size, irAEs may sometimes be rare (40, 41) or even absent. To address this issue, we used frequentists-framework-based network meta-analyses for all statistical analyses, and if there were no irAEs observed in a specific arm of a trial, the classic continuity correction of 0.5 for zero cells was applied for data preparation (26). Treatment effects were reported as the surface under the cumulative ranking curves (SUCRA). The higher SUCRA scores indicates a higher risk of irAEs.

Because consistency assessment is crucial in ensuring the robustness of direct and indirect comparison results (42), we used a two-step method to evaluate consistency. First, we used the loop-specific approach to evaluate the presence of inconsistency from direct and indirect evidence (43). We calculated the inconsistency factors (IF) values, standard error of inconsistency factors (seIF) and p-value. If the 95% CI of IF contained '0' and the p-value was higher than '0.05', it was considered the direct evidence to be consistent with the indirect evidence. Second, we adopted node-splitting models to identify the consistency in the entire network on particular comparisons (nodes). $P>0.05$ indicated no significant inconsistency.

To evaluate the transitivity of the NMA, we compared the distribution of patient characteristics, aiming to ensure the similarity of the distribution of effect modifiers across different treatment comparisons in the network of trials. Furthermore, "comparison-adjusted" funnel plots were utilized to assess the

presence small-sample effect and publication bias within the network of interventions.

3 Results

3.1 Study selection and patient characteristics

We identified 3 604 citations up to August 15, 2022, including 325 records from PubMed, 464 from Embase, 43 from Web of Science, 2 543 from Cochrane and 229 from ClinicalTrials.gov (Figure 1). After removing duplicates, 3 112 records were included in the titles and abstracts screening. A total of 641 publications underwent full-text review, after excluding 2 471 publications. Twenty-two RCTs (44–65) met the study selection criteria and were included in the analysis. Figure 2 shows that among the patients included in the network meta-analysis, 3 919 patients received ICIs, 1 386 patients received ICI+nab-PTX, 3 302 patients received ICI+PTX, and 7 356 patients received chemotherapy.

Table 1 show the information on the baseline characteristics of the included trials. There were 20 two arm trials, and only 2 studies have three arms. Supplementary Table 2 displays the occurrence of irAEs in different treatment groups. all studies were phase III trials. Cancer types tested in these studies included lung cancer (n=12), breast cancer (n=3), urothelial cancer (n=3), ovarian cancer (n=2), gastric cancer (n=2). More detailed information can be found in Supplementary Table 1.

Figure 3 presents the risk of bias summary for the included trials. It is worth noting that many trials were open-labeled due to the differences in infusion duration, administration schedules, and premedication requirements for immune checkpoint inhibitors, which would make masking difficult, but this does not indicate that the studies were of low quality.

3.2 Heterogeneity, inconsistency, and transitivity assessment

Pairwise comparisons with heterogeneity estimates are presented in Figure 4 and Supplementary Figure 1. Nearly all comparisons indicating low heterogeneity. Inconsistency analysis using node-splitting and loop-specific approaches showed no significant inconsistency between direct and indirect analyses. Results of the inconsistency evaluation are presented in Supplementary Tables 6, 7. All included clinical trials enrolled cancer patients; all the trials were phase III RCTs; utilizing standard doses (the dosage of PTX in the Asian population was 175 mg/m^2/3 weeks, and of other races was 200mg/m^2/3 weeks); median follow-up time was 23.2 months (ranging from 7.9 to 60 months); patients were at advanced stages of cancer, PS scores were mostly 0-1, and age characteristics were similar. By comparison, the

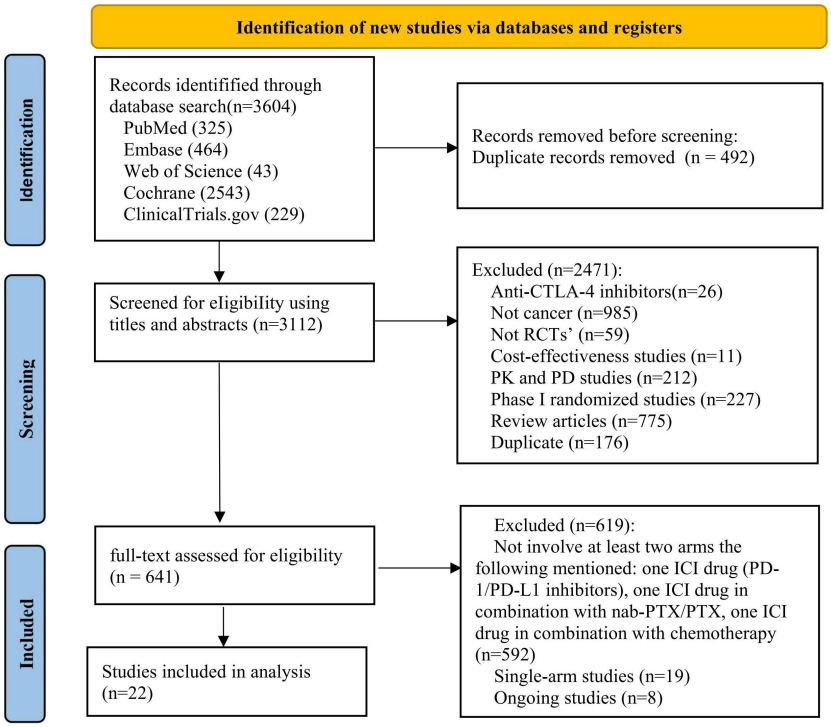

FIGURE 1
Flowchart of study selection followed PRISMA guidelines.

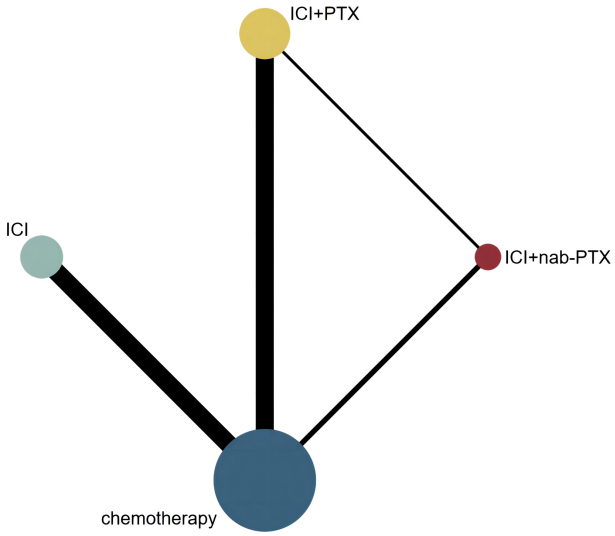

FIGURE 2
Network geometry of any event. The nodes in the figure represent the interventions being compared, while the edges represent the direct comparisons available between pairs of interventions (i.e. comparisons evaluated in at least one study). The node sizes are weighted based on the number of patients in each intervention arm, while the edges are weighted using inverse variance.

baseline characteristic distribution of each treatment group was balanced, indicating acceptable transitivity. The network's funnel plots visually indicate potential publication bias, and no significant asymmetry was observed (Supplementary Figure 6)

3.3 Comparison of irAEs

The network geometry and the contribution plots are reported in Supplement Figures 2, 3. While head-to-head direct meta-analyses are shown in Figure 4 and Supplementary Figure 1.

Based on the consistency model, the ORs for pairwise comparisons of irAEs are shown in Table 2. Almost all treatment regimens showed statistically significant differences with the chemotherapy group. The ICI+PTX had a significantly lower risk of grade 1-5 hyperthyroidism (OR=0.46, 95% CrI: 0.22-0.96) and grade 1-5 hypothyroidism (OR=0.49, 95% CrI: 0.26-0.93) than ICIs. Notably, comparing with ICI monotherapy, ICI+nab-PTX was associated with a decreased risk of grade 3-5 pneumonitis (OR=0.28, 95% CrI: 0.09,0.90).

The ranking analysis performed with SUCRA provided a ranking of each treatment group based on the incidence of irAEs, as shown in Table 3. The ICIs was associated with the worst safety ranking for grade 1-5 of any event (probability=79.3%), followed by ICI+PTX (70.8%), ICI+nab-PTX (49.9%), and finally chemotherapy (0%). The safety ranking for grade 3-5 of any adverse event was the same to irAEs: ICIs (84.6%), ICI+PTX (76.8%), ICI+nab-PTX (38.6%), and chemotherapy (0%). In addition, compared to the other three treatment groups, ICI monotherapy had the highest risk for causing pneumonitis (grade 1-5 and grade 3-5), colitis (grade 1-5), hepatitis (grade 1-5 and grade 3-5), hypothyroidism (grade 1-5) and hyperthyroidism (grade 3-5). The main irAEs caused by ICI+PTX was rare. ICI+nab-PTX mainly caused grade 3-5 colitis. Additionally, ICIs+nab-PTX has a lower risk of irAEs than ICIs +PTX. More detailed information can be found in Supplementary Table 4 and Supplementary Figures 4, 5.

3.4 Comparison of irAEs between PD-1 and PD-L1

The network geometry and the contribution plots are reported in Supplement Figures 2, 3. While head-to-head direct meta-analyses are shown in Supplementary Figure 1. Supplementary Table 3 presents the safety profiles of six treatment groups. For grade 1-5 irAEs, all treatment groups were associated with statistically significantly higher risks compared with chemotherapy. PD-1+PTX was associated with a statistically significantly lower risk of pneumonitis compared to PD-1 (OR=0.32, 95% CrI: 0.11-0.92). PD-L1+PTX showed a noticeably lower risk of rash compared to PD-1+PTX (OR=0.52, 95% CrI: 0.28-0.98). Additionally, PD-L1 +PTX also presented a lower risk of hypothyroidism compared to PD-L1(OR=0.34, 95% CrI: 0.12-1.00). Of note, adding PTX to the treatment regimens can increase the risk of pneumonitis and hypothyroidism. Secondly, PD-1 showed a significantly higher risk than PD-L1.

Statistically substantial differences were observed only when comparing with chemotherapy groups for grade 3-5 irAEs. The ranking probability is presents in Table 3B, Supplementary Table 5 and Supplementary Figure 4, 5. The treatment groups containing PD-1 exhibited a higher risk of adverse reactions than those containing PD-L1.

TABLE 1 Baseline characteristics of 22 studies.

First author, year	Patients	PD-1/ PD-L1	Treatment	Dosage	Stage	Line	Phase	Sample size	Median follow-up time (months)	Edition of CTCAE	PS 0-1	Famale	Age
Socinski MA, 2018	NSCLC	PD-L1	Ate+Bev+PTX + CBP	Ate (1200mg/ 3weeks)	IV	1	3	393	15.4	CTCAE 4.0	400	160	63
			Bev+PTX+CBP	PTX (200mg/ m2/3weeks				394			400	161	63
Sugawara S, 2021	NSCLC	PD-1	Niv+Bev+PTX + CBP	Niv (360mg/ 200mg/m2)	IIIB/IV	1	3	273	13.7	CTCAE 4.0	275	70	64.1
			Placebo+Bev+PTX + CBP	PTX (200mg/ m2/3weeks)				275			275	69	65.1
West H, 2019	NSCLC	PD-L1	Ate+Nab-PTX+CBP	Ate (1200mg/ 3weeks)	IV	1	3	473	18.5	CTCAE 4.0	482	206	66.1
			nab-PTX+CBP	nab-PTX (100mg/we)				232			239	102	67.1
Jotte R, 2020	NSCLC	PD-L1	Ate+CBP+nab-PTX	Ate (1200mg/ 3weeks)	IV	1	3	334	18.1	CTCAE 4.0	342	63	68.1
			Ate+CBP+PTX	PTX (200mg/ m2/3weeks)				332			338	60	69.1
			CBP+nab-PTX	nab-PTX (100mg/we)				334			339	63	70.1
Moore KN, 2021	Ovarian Cancer	PD-L1	Ate+PTX+CBP+Bev	Ate (1200mg/ 3weeks)	III/IV	frontline	3	642	19.9	CTCAE 4.0		642	73.1
			Placebo+PTX+CBP+Bev	PTX (175mg/ m2/3weeks)				644				644	74.1
Wang J, 2021	NSCLC	PD-1	Tis+CBP+PTX	Tis (200mg/ 3weeks)	IIIB/IV	1	3	120	8.6	CTCAE 5.0	120	13	75.1
			Tis+CBP+nab-PTX	nab-PTX (100mg/we)				119			119	7	76.1
			CBP+PTX	PTX (175mg/ m2/3weeks)				121			121	10	77.1
Monk BJ, 2021	Epithelial Ovarian Cancer	PD-1	Ave+PTX+CBP	Ave (10mg/kg/ 2weeks)	III/IV	frontline	3	329	29	CTCAE 4.0	329	331	80.1
			PTX+CBP	PTX (175mg/ m2/3weeks)				334			332	335	81.1
Hellmann MD, 2018	NSCLC	PD-1	Nivoluma	Niv (240mg/ 2weeks)	IV	1	3	391	min 11.2	CTCAE 4.0	394	124	64

(Continued)

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TABLE 1 Continued

First author, year	Patients	PD-1/ PD-L1	Treatment	Dosage	Stage	Line	Phase	Sample size	Median follow-up time (months)	Edition of CTCAE	PS 0-1	Famale	Age
			Platinum doublet chemotherapy					570			577	198	64
Herbst RS, 2020	NSCLC	PD-L1	Ate	Ate (1200mg/ 3weeks)	IV	1	3	286	54.5	CTCAE 4.0	285	87	64
			platinum-based chemotherapy					263			287	89	65
Powles T, 2021	Urothelia Cancer	PD-1	Pem	Pem (200mg/ 3weeks)	locally advanced, unresectable, or metastati	1	3	302	31.7	CTCAE 4.0	282	79	67
			Standard-of-care Chemotherapy					342			330	90	68
Powles T, 2020	Urothelia Cancer	PD-L1	Dur	Dur (1500mg/ 4weeks)	unresectable, locally advanced or metastati	1	3	345	41.2	CTCAE 4.0	346	97	67
			Standard of Care Chemotherapy					313			344	70	68
Sezer A, 2021	NSCLC	PD-1	Cem	Cem (350mg/ 3weeks)	IIIB/IIIC/IV	1	3	355	32	CTCAE 4.0	356	44	63
			Standard-of-care Chemotherapy					342			354	60	64
Rizvi NA, 2020	NSCLC	PD-L1	Dur	Dur (20mg/kg/ 4weeks)	IV	1	3	369	40	CTCAE 4.0	372	118	63.2
			Standard-of-care Chemotherapy					352			370	122	63.6
Shitara K, 2020	Gastric Cancer	PD-1	Pem	Pem (200mg/ 3weeks)	locally advanced/ unresectable or metastati	1	3	254	29.4	CTCAE 4.0	265	76	61
			Placebo +Standard-of-care Chemotherapy					244			250	71	62.5
Reck M, 2021	NSCLC	PD-1	Pem	Pem (200mg/ 3weeks)	IV	1	3	154	60		153	62	64.5
			platinum-based chemotherapy					151			151	56	66
Mok TSK, 2019	NSCLC	PD-1	Pem	Pem (200mg/ 3weeks)	locally advanced or metastati	1	3	636	12.8	CTCAE 4.0	637	187	63
			platinum-based chemotherapy					615			637	185	63

(Continued)

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TABLE 1 Continued

First author, year	Patients	PD-1/ PD-L1	Treatment	Dosage	Stage	Line	Phase	Sample size	Median follow-up time (months)	Edition of CTCAE	PS 0-1	Famale	Age
Carbone DP, 2017	NSCLC	PD-L1	Niv	Niv (3mg/kg/ 2weeks)	IV	1	3	267	13.5	CTCAE 4.0	268	87	63
			platinum-based chemotherapy					263			269	122	65
Emens LA, 2021	Triple-Negative Breast Cancer	PD-L1	Ate plus nab-PTX	Ate (840mg/2 weeks)	locally advanced, or metastati	1	3	460	18.8	CTCAE 4.0	450	448	55
			nab-PTX+ placebo	nab-PTX (100mg/we)				430			450	450	56
Miles D, 2021	Triple-Negative Breast Cancer	PD-L1	Ate+PTX	Ate (840mg/ 2weeks)	metastatic or unresectable locally advance	1	3	432	14.2	CTCAE 4.0	431	430	54
			placebo+PTX	PTX (90 mg/ m2/we)				217	14.5		220	220	53
Schmid P, 2020	Triple-Negative Breast Cancer	PD-1	Pem+ PTX+ CBP	Pem (200mg/ 3weeks)	II/III	1	3	781	15.5	CTCAE 4.0	784	783	49
			Placebo+ PTX+CBP	PTX (80 mg/ m2/we)				389			390	390	48
Shitara K, 2018	gastric or gastro-oesophageal junction cancer	PD-1	Pem	Pem (200mg/ 3weeks)	advance	2	3	294	7.9	CTCAE 4.0	296	94	62.5
			PTX	PTX (standard-dos)				276			295	88	60
Bellmunt J, 2017	Urothelial Cancer	PD-1	Pem	Pem (200mg/ 3weeks)	advance	2	3	266	14.1	CTCAE 4.0	262	70	67
			Chemotherapy					255			264	70	65

NSCLC, non-small cell lung cancer; PTX, Paclitaxel; nab-PTX, nanoparticle albumin-bound paclitaxel; Ate, Atezolizumab; Tis, Tislelizumab; AVE, Avelumab; Niv, Nivolumab; Pem, Pembrolizumab; Dur, Durvalumab; Cem, Cemiplimab; Bev,Bevacizumab; CBP, Carboplatin.

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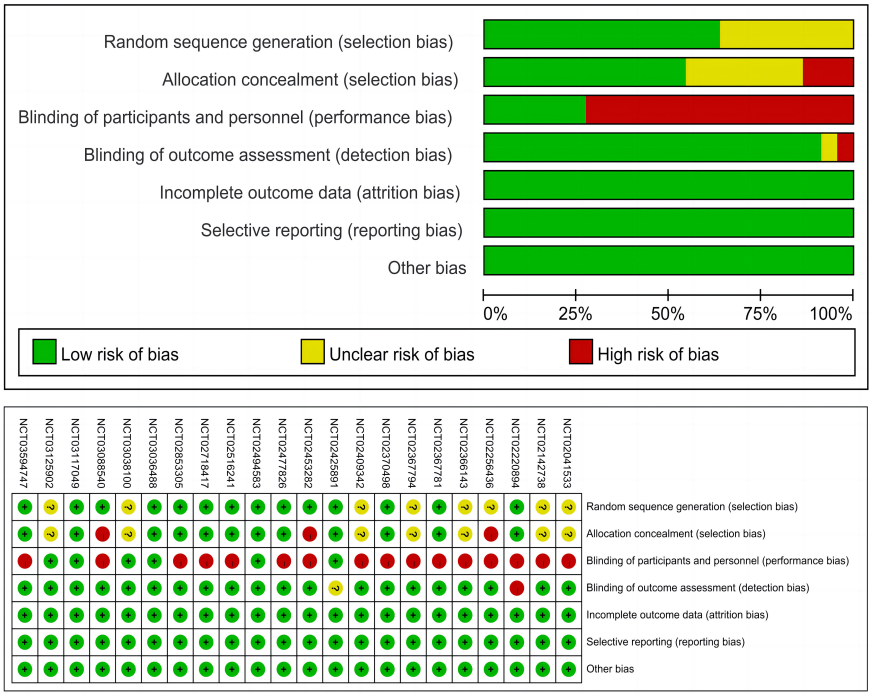

FIGURE 3
Risk of bias assessment for the 22 included randomized controlled trials.

4 Discussion

Immunotherapy has revolutionized treatment approaches for cancer, with ICIs combined with chemotherapy have shown remarkable clinical benefits, particularly the wide used nab-PTX/PTX combination strategies (13, 66). This large NMA is based on 22 RCTs including 15 963 patients. To our knowledge, this study is the first NMA that includes all cancers and provide important safety ranking of four treatment regimens involving ICI+ nab-PTX/PTX and a comparison of the safety profiles between PD-1 and PD-L1 inhibitors are provided as valuable references for clinical practice.

The nab-PTX was associated with unique advantages, such as without the use of a solvent, faster and greater tissue penetration, and slower elimination compared to PTX, which has made nab-PTX as the preferred option for combination therapy in the clinical settings (13). However, some studies have indicated that immunetherapy+chemotherapy may decrease the risk of irAEs (32, 33), while others have reached the opposite conclusion (32, 33, 67, 68). In our analysis, we found that the specific combination regimen of nab-PTX/PTX+ICIs is a safer therapeutic approach, significantly reducing the risk of irAEs occurrence. Moreover, nab-PTX demonstrates superior safety compared to PTX

The immune-related pneumonitis was associated with treatment discontinuation and mortality (69, 70). In this analysis, we specifically evaluated the immune-related pneumonitis. We found ICI monotherapy was linked to a higher risk of grade 3-5 immune-related pneumonitis compared to nab-PTX+ICI, and comparing with PD-1, PD-1+PTX was associated with a statistically significant lower risk of grade 1-5 pneumonitis. In addition, ICI therapy was found to be associated with increased risks of grade 1-5 hypothyroidism and hyperthyroidism compared with PTX+ICI, while PD-L1+PTX presented a lower risk of hypothyroidism compared to PD-L1. In addition, according to the ranking results of irAEs, nab-PTX/PTX+ICI has a better safety profile than ICIs monotherapy for most irAEs, potentially reducing the risk of irAEs associated with ICIs. One possible reason is that in phase III clinical trials, the use of cytotoxic chemotherapy drugs is close to the maximum tolerated dose, leading to immune-suppressive (71). Consequently, the incidence of adverse events is low. Additionally, patients receiving PTX need to be pretreated with corticosteroids to prevent hypersensitivity (72), which can also suppress the immune system, reduce inflammation, and alleviate the development of irAEs (73). There may be other underlying mechanisms contributing to this phenomenon that require further investigation in basic research. In summary, our findings suggest that combining nab-PTX/PTX with ICIs offers a safer clinical treatment strategy.

For immune-related rash, PD-1+PTX was found to significantly increase the risk of grade 1-5 rash compared with PD-L1+PTX. In addition, according to the ranking of adverse reactions, our analysis found that the group containing PD-1 had s higher risk of irAEs compared to the group containing PD-L1, which is consistent with the previous research results (32, 74–76). In contrast, PD-1 antibody can simultaneously block the binding of PD-1 to both PD-L1 and PD-L2, resulting in a more comprehensive inhibition of the immune escape pathway and a higher incidence of irAEs (77). A previous study has reported that the competitive binding of PD-1 antibody to PD-L2 can disrupt the normal function of PD-L2 and other binding partners, leading to the activation of RGMb (repulsive guidance molecule) and subsequently cause pneumonitis (78). A recent study has demonstrated that

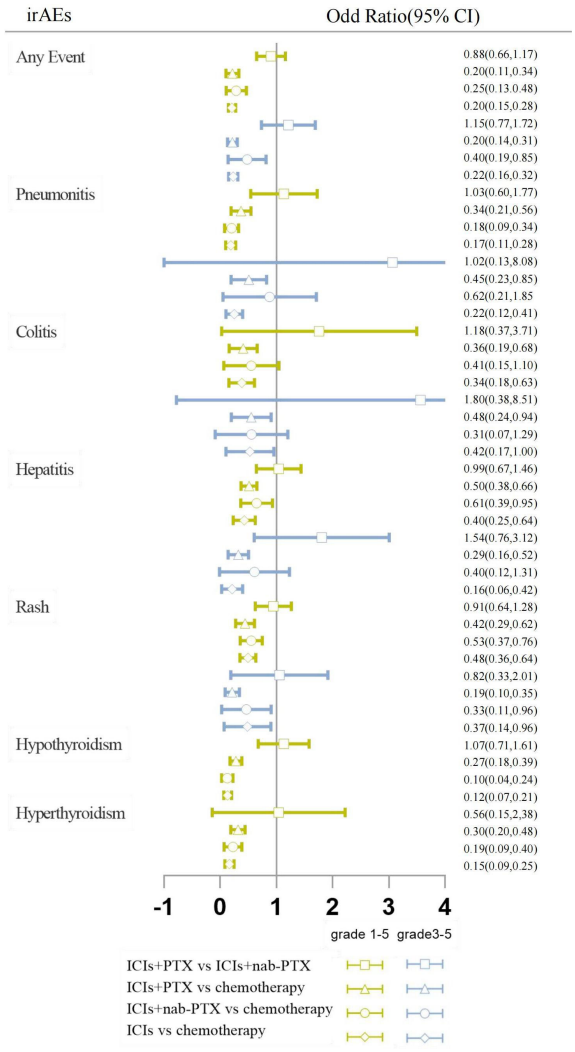

FIGURE 4
Forest plots results of head-to-head comparisons. The results are presented as odds ratios (ORs) with corresponding 95% confidence intervals (CIs). The vertical line represents the null effect, which is set at 1. The horizontal line depicts the CIs, and the hollow shape represents the point estimate, summarizing the ORs. When interpreting the forest plot for each pairwise comparison, it should be noted that if the hollow shape with the CI does not intersect with the vertical line of null effect, a statistically significant difference is observed. If the CI is on the left of the null effect, the event is significantly higher in the intervention arm, while if the CI is on the right, the event is statistically more frequent in the reference arm. If the CI intersects with the line of null effect, the difference between the two procedures is not statistically significant.

exosomes derived from melanoma cells also express PD-L1. These exosomes, which contain PD-L1, travel through the bloodstream and can directly bind to the PD-1 receptor on the surface of T cells, resulting in T cell dysfunction. As a result, PD-L1 antibodies may be rendered ineffective by exosomal-PD-L1 before reaching the tumor cells. However, this issue does not arise with PD-1 antibodies, as they bind to the PD-1 receptor on T cells and exosomal-PD-L1 cannot neutralize their effects (79). These reasons may all contribute to the enhanced safety of PD-L1 inhibitors compared to PD-1 inhibitors. Numerous basic studies have reported a synergistic effect of taxane-combined immunotherapy (29, 80–82). This study further supports the potential benefits of this strategy in reducing the incidence of irAEs, providing a valuable guidance for clinical decision-making and serving as an evidence-based foundation for further basic research. However, there is still a lack of evidence of direct comparison. Therefore, further prospective RCTs and detailed basic research are needed to enrich the evidence.

Our study has several limitations. Frist, a standardized diagnostic criteria for irAEs is still lacking. In this study, irAEs data were extracted from “immune-related adverse events”. To obtain a more robust estimate of safety profile, we also extracted “treatment-related adverse events. In addition, although CTCAE 4.0 was the main version of adverse event evaluation criteria in all trials, we could not exclude the possibility that the different judgment criteria and grading strategies have been applied in the evaluation of irAEs. Second, the median follow-up time was varied among the trials included in the analysis, and it is possible that the reporting of irAEs with late-onset might be varied greatly. Third, the expression level of PD-L1 has been recognized as a potentially important and clinically valuable indicator for anti-PD-1 or anti-PD-L1 treatment

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TABLE 2 The odds ratios (ORs) for pairwise comparisons of irAEs based on network consistency model.

Grade 3-5 Pneumonitis				Grade 3-5 Colitis			
ICI+nab-PTX	1.46 (0.59,3.60)	**3.61 (1.12,11.67)**	0.68 (0.26,1.74)	ICI+nab-PTX	0.57 (0.17,1.87)	0.58 (0.13,2.65)	**0.26 (0.08,0.87)**
1.50 (0.70,3.20)	ICI+PTX	2.48 (0.99,6.21)	**0.46 (0.25,0.85)**	0.89 (0.61,1.31)	ICI+PTX	1.02 (0.33,3.18)	**0.47 (0.25,0.88)**
0.73 (0.28,1.87)	0.49 (0.22,1.07)	ICI	**0.19 (0.09,0.37)**	0.65 (0.33,1.29)	0.73 (0.39,1.37)	ICI	0.46 (0.18,1.16)
5.05 (2.37,10.78)	**3.37 (1.97,5.77)**	**6.93 (3.89,12.35)**	chemotherapy	**1.71 (1.21,2.42)**	**1.92 (1.42,2.60)**	**2.64 (1.51,4.61)**	chemotherapy
Grade 1-5 Pneumonitis				Grade 1-5 Colitis			
Grade 3-5 Hepatitis				Grade 3-5 Rash			
ICI+nab-PTX	1.11 (0.43,2.86)	2.17 (0.55,8.56)	**0.34 (0.14,0.79)**	ICI+nab-PTX	1.43 (0.64,3.20)	0.76 (0.20,2.92)	**0.24 (0.10,0.57)**
0.89 (0.61,1.31)	ICI+PTX	1.96 (0.53,7.32)	**0.30 (0.14,0.64)**	0.85 (0.55,1.31)	ICI+PTX	0.53 (0.17,1.69)	**0.17 (0.10,0.28)**
0.65 (0.33,1.29)	0.73 (0.39,1.37)	ICI	**0.15 (0.05,0.45)**	0.89 (0.51,1.57)	1.05 (0.65,1.71)	ICI	**0.32 (0.11,0.89)**
1.71 (1.21,2.42)	**1.92 (1.42,2.60)**	**2.64 (1.51,4.61)**	chemotherapy	**1.88 (1.25,2.85)**	**2.23 (1.68,2.96)**	**2.11 (1.43,3.12)**	chemotherapy
Grade 1-5 Hepatitis				Grade 1-5 Rash			
Grade 1-5 Hyperthyroidism				Grade 1-5 Any Event			
ICI+nab-PTX	0.95 (0.50,1.80)	2.08 (0.89,4.90)	**0.26 (0.13,0.50)**	ICI+nab-PTX	1.23 (0.62,2.44)	1.33 (0.63,2.85)	**0.26 (0.14,0.48)**
1.48 (0.80,2.75)	ICI+PTX	**2.20 (1.04,4.64)**	**0.27 (0.16,0.46)**	0.65 (0.36,1.18)	ICI+PTX	1.08 (0.56,2.08)	**0.21 (0.13,0.34)**
0.72 (0.33,1.57)	**0.49 (0.26,0.93)**	ICI	**0.13 (0.07,0.22)**	0.59 (0.28,1.24)	0.91 (0.47,1.77)	ICI	**0.19 (0.12,0.31)**
6.04 (3.08,11.85)	**4.09 (2.61,6.40)**	**8.39 (4.98,14.15)**	chemotherapy	**2.90 (1.67,5.03)**	**4.48 (2.90,6.92)**	**4.93 (2.96,8.20)**	chemotherapy
Grade 3-5 Hyperthyroidism				Grade 3-5 Any Event			

This is an indirect comparison of adverse events of grades 1-5 and 3-5 in different treatment regimens. The combined odds ratios and 95% confidence intervals indicate the results between the highest and lowest treatment regimens. Each unit contains the combined odds ratio and 95% confidence interval, with significant results highlighted in thick lines.

TABLE 3 Pooled results of toxicity spectra and SUCRA rankings based on each specific irAEs.

(A)	1	2	3	4
Grade 1-5 Any Event	ICI (79.3)	ICI+PTX (70.8)	ICI+nab-PTX (49.9)	Chemotherapy (0.0)
Grade 3-5 Any Event	ICI (84.6)	ICI+PTX (76.8)	ICI+nab-PTX (38.6)	Chemotherapy (0.0)
Grade 1-5 Pneumonitis	ICI (90.4)	ICI+nab-PTX (70.2)	ICI+PTX (39.4)	Chemotherapy (0.0)
Grade 3-5 Pneumonitis	ICI (98.6)	ICI+PTX (60.6)	ICI+nab-PTX (33.6)	Chemotherapy (7.3)
Grade 1-5 Colitis	ICI (78.1)	ICI+nab-PTX (64.6)	ICI+PTX (57.0)	Chemotherapy (0.3)
Grade 3-5 Colitis	ICI+nab-PTX (85.4)	ICI (57.3)	ICI+PTX (54.8)	Chemotherapy (2.5)
Grade 1-5 Hepatitis	ICI (91.2)	ICI+PTX (62.9)	ICI+nab-PTX (45.8)	Chemotherapy (0.1)
Grade 3-5 Hepatitis	ICI (90.4)	ICI+PTX (57.9)	ICI+nab-PTX (51.4)	Chemotherapy (0.2)
Grade 1-5 Rash	ICI+PTX (78.3)	ICI (69.5)	ICI+nab-PTX (52.2)	Chemotherapy (0.0)
Grade 3-5 Rash	ICI+PTX (88.9)	ICI+nab-PTX (61.5)	ICI (49.2)	Chemotherapy (0.5)
Grade 1-5 Hypothyroidism	ICI (92.8)	ICI+nab-PTX (69.8)	ICI+PTX (37.5)	Chemotherapy (0.0)

(Continued)

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TABLE 3 Continued

(A)	1	2	3	4		
Grade 1-5 Hyperthyroidism	ICI (97.8)	ICI+nab-PTX (53.6)	ICI+PTX (48.6)	Chemotherapy (0.0)		
(B)	**1**	**2**	**3**	**4**	**5**	**6**
Grade 1-5 Any Event	PD-1+PTX (87.0)	PD-1 (69.7)	PD-L1 (58.6)	ICI+nab-PTX (45.9)	PD-L1 +PTX (39.5)	Chemotherapy (0.0)
Grade 3-5 Any Event	PD-1+PTX (87.0)	PD-L1 (73.0)	PD-1 (65.7)	PD-L1 +PTX (43.1)	ICI+nab-PTX (31.2)	Chemotherapy (0.0)
Grade 1-5 Pneumonitis	PD-1 (96.4)	ICI+nab-PTX (68.5)	PD-L1 (48.7)	PD-L1 +PTX (47.4)	PD-1 +PTX (38.8)	Chemotherapy (0.3)
Grade 3-5 Pneumonitis	PD-1 (90.6)	PD-L1+PTX (65.7)	PD-L1 (64.8)	PD-1 +PTX (40.7)	ICI+nab-PTX (34.1)	Chemotherapy (4.0)
Grade 1-5 Colitis	PD-L1 (79.8)	PD-1+PTX (77.9)	PD-1 (57.7)	ICI+nab-PTX (48.5)	PD-L1 +PTX (35.2)	Chemotherapy (1.0)
Grade 3-5 Colitis	PD-1+PTX (87.6)	ICI+nab-PTX (65.2)	PD-L1 (54.2)	PD-1 (54.0)	PD-L1 +PTX (31.5)	Chemotherapy (7.5)
Grade 1-5 Hepatitis	PD-1 (90.2)	PD-1+PTX (79.0)	PD-L1 (52.7)	PD-L1 +PTX (45.8)	ICI+nab-PTX (31.7)	Chemotherapy (0.6)
Grade 3-5 Hepatitis	PD-1+PTX (74.4)	PD-1 (73.3)	PD-L1 (70.3)	ICI+nab-PTX (41.9)	PD-L1 +PTX (39.0)	Chemotherapy (1.2)
Grade 1-5 Rash	PD-1+PTX (94.9)	PD-1 (67.0)	PD-L1 (49.1)	PD-L1 +PTX (46.2)	ICI+nab-PTX (42.7)	Chemotherapy (0.2)
Grade 3-5 Rash	PD-1+PTX (88.5)	PD-1 (70.2)	PD-L1+PTX (57.2)	ICI+nab-PTX (51.0)	PD-L1 (31.0)	Chemotherapy (2.2)
Grade 1-5 Hypothyroidism	PD-L1 (87.8)	PD-1 (75.0)	ICI+nab-PTX (62.1)	PD-1 +PTX (47.9)	PD-L1 +PTX (27.2)	Chemotherapy (0.0)
Grade 1-5 Hyperthyroidism	PD-1 (89.6)	PD-L1 (71.8)	PD-1+PTX (67.3)	ICI+nab-PTX (42.7)	PD-L1 +PTX (28.5)	Chemotherapy (0.0)

The number in each bracket indicates the probability of risk ranking. (A) If ICI+nab-PTX has a higher ranking than ICI+PTX, the squares are shown with a yellow background. Otherwise, they are displayed on a blue background. (B) If the treatment groups containing PD-1 have a higher ranking than those with PD-L1, the squares are shown with a yellow background. Otherwise, they are displayed on a blue background.

(83, 84), but most trials failed to provide this important information. Fourth, the limited sample size of arms containing nab-PTX also prevents subgroup analysis, and the results involving nab-PTX should be interpreted with caution. Finally, for zero-events in any arm, STATA replaced them with the default value of 0.5, which increased the sample size per treatment by 1.

5 Conclusion

Our findings demonstrate that this combination therapies can significantly reduce the risk of immune-related adverse events, providing robust evidence to address the current controversial academic issues and offering clinical decision-making guidance for cancer patients. Furthermore, this study also confirms previous research findings that anti-PD-L1 inhibitors are safer than anti-PD-1 inhibitors and it demonstrates ICIs+nab-PTX has a lower risk of irAEs occurrence than ICIs+PTX.

Author contributions

Conception and design: WH, JY, and WC. Wrote the manuscript: WH. Acquired data: WH, JZ and YW. Analyzed the

data: WH. Discussed the results and implications of findings: WH, JZ, YW, BF, and SJ. Drafting of the manuscript: WH. Review and editing: JY and WC. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2023.1175809/full#supplementary-material

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Severe thyrotoxicosis induced by tislelizumab: a case report and literature review

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Immune checkpoint inhibitors (ICIs) have made significant breakthroughs in the treatment of a variety of malignancies. As its use increases, the unique immune-mediated toxicity profile of ICIs are becoming apparent. We report a case of immune-related endocrine adverse events (irAE) in a patient with hepatocellular carcinoma treated with anti-programmed cell death protein 1 (PD-1) (tislelizumab). Although many irAEs have been reported, few cases of severe thyrotoxicosis have been described after immunotherapy in the literature. We present the case of a 49-year-old male who experienced a Grade 3 tislelizumab-related adverse reaction according to Common Terminology Criteria for Adverse Events (CTCAE5.0) and received methylprednisolone, thiamazole, and levothyroxine sodium tablets. Early identification of irAEs, risk factors, regular monitoring, use of steroids and/or immunoglobulins, and adjuvant supportive care are critical to the clinical prognosis of patients. It should be underlined that the tumor benefits of ICI therapy outweigh the risks associated with ICI-induced endocrine disorders, and ICI treatment should not be stopped or delayed except in rare cases (adrenal crisis, severe thyrotoxicosis). The familiarity of healthcare professionals with irAEs of the thyroid when thyrotoxicosis occurs is important to facilitate an effective diagnosis and appropriate treatment of this increasingly common thyroid disorder.

KEYWORDS

tislelizumab, immune-related adverse events, hyperthyroidism, hypothyroidism, thyroiditis

Introduction

Immunotherapy has made a significant breakthrough and has evolved to be a standard treatment regimen in cancer treatment since the 1990s (1). In recent years, ICIs have emerged as a powerful class of immunotherapeutic medicine and are now approved for advanced malignancies by the US Food and Drug Administration. Monoclonal antibodies

targeting ICIs mainly block negative regulators of T cell activation by targeting cytotoxic T lymphocyte-associated antigen 4 (CTLA-4): ipilimumab, programmed cell death 1 (PD-1) (nivolumab, pembrolizumab, cemiplimab), and its programmed cell death ligand 1 (PD-L1) (durvalumab, atezolizumab, avelumab) (2–4). PD-1 is a negative regulator of T cell activity and, when it interacts with its two ligands PD-L1 and PD-L2, it can limit T cell activation at various stages of the immune response. PD-1 plays a key role in tumor evasion of host immunity (5). Given their mechanism of action, it understandable that ICIs can also trigger autoimmune side effects or immune-related adverse events (irAEs) in recipients (6). These irAEs can affect multiple systems and sites, including the gastrointestinal tract, endocrine, skin, and liver (7). Among them, thyroid dysfunction (TD) is the most common endocrine irAEs, and mainly includes hyperthyroidism, hypothyroidism, and thyroiditis.

Tislelizumab is a new humanized IgG4 PD-1 inhibitor that was approved by the National Medical Products Administration (NMPA) in China for the treatment of classical relapsed or refractory Hodgkin lymphoma after at least second-line systemic chemotherapy, locally advanced or metastatic urothelial cancer, non-small cell lung cancer, and hepatocellular carcinoma in December 2019. Several clinical trials involving multiple indications for tislelizumab are ongoing (Table 1).

The main side effects related to treatment are irAEs, including rash, pruritus, thyroiditis, diarrhea, hepatitis, and pneumonitis. Grade 3 and higher adverse effects caused by tislelizumab include severe skin reactions, anemia, pneumonitis, hypertension, and adrenocortical insufficiency (8–10). However, cases of autoimmune Grade 3 and higher hypothyroidism induced by immunotherapy are rare and have been poorly described. We describe a case of severe hypothyroidism induced by tislelizumab.

Case presentation

A middle-aged 49-year-old male underwent a routine physical examination in a local hospital 5 days before admission. Abdominal color ultrasound showed a solid intrahepatic mass (9 October, 2021). Laboratory results revealed alpha-fetoprotein 1.56 ng/mL, carcinoembryonic antigen 2.56 ng/mL, alanine aminotransferase 35 U/L, aspartate aminotransferase 147 U/L, direct bilirubin 34 μmol/L, and indirect bilirubin 11 μmol/L. Abdominal computed tomography (CT) revealed space-occupying lesions that involved the right lobe of the liver, with a small amount of bleeding. A subsequent upper abdominal plain scan and enhanced CT performed in our hospital (11 October) confirmed space-occupying lesions in the right lobe of the liver, about 13.5×13.9 cm in size, suggestive of liver cancer (Figure 1). Laboratory tests showed hepatitis B virus surface antigen(+) and hepatitis B virus core antibody(+). During this period, the patient had no abdominal pain, abdominal distension, nausea, vomiting, or other discomfort. He had a history of hypertension of more than 3 years, regular oral administration of perindopril tert-butylamine and bisoprolol fumarate, and a history of coronary heart disease of 3 years. He underwent a coronary stent implantation at another institution 3

TABLE 1 Key ongoing clinical trials involving tislelizumab.

Trial name	Indication	Phase	Status	NCT
Surufatinib in combination with tislelizumab in subjects with advanced solid tumors	Metastatic solid tumor	I/II	Recruiting	NCT04579757
Safety and efficacy study of tislelizumab in combination with BCG in HR-NMIBC patients (TACBIN-01)	Urinary bladder neoplasms	I/II	Recruiting	NCT04922047
A study to evaluate the safety and efficacy of oral APL-1202 in combination with tislelizumab compared to tislelizumab alone as neoadjuvant therapy in patients with muscle invasive bladder cancer	Muscle invasive bladder cancer	I/II	Recruiting	NCT04813107
Chemoradiation plus tislelizumab for conversion therapy of locally nonresectable ESCC	Esophageal squamous cell carcinoma	I/II	Recruiting	NCT05394415
Study of tislelizumab in combination with Oxaliplatin and Tegafur for the treatment of gastric cancer with liver metastases	Liver metastases	II/III	Recruiting	NCT05325528
Tislelizumab in combination with lenalidomide in refractory and relapsed older adult patients with non-GCB DLBCL	Non-GCB/ABC diffuse large B-cell lymphoma	I/II	Recruiting	NCT04796857
Phase I/IIa study of BR790 in combination with tislelizumab in adult subjects with advanced solid tumors	Advanced solid tumor	I/II	Recruiting	NCT05505877
Tislelizumab combined with mitoxantrone hydrochloride liposome in extranodal natural killer/T cell lymphoma	Extranodal natural killer T cell lymphoma	I/II	Recruiting	NCT05464433
GEMOX combined with donafenib and tislelizumab in biliary tract cancer	Biliary tract carcinoma	I/II	Recruiting	NCT04979663
Adjuvant PD-1 antibody in combination with capecitabine for patients with ICC at high-risk of postoperative recurrence	Cholangiocarcinoma, intrahepatic	I/II	Recruiting	NCT04782804

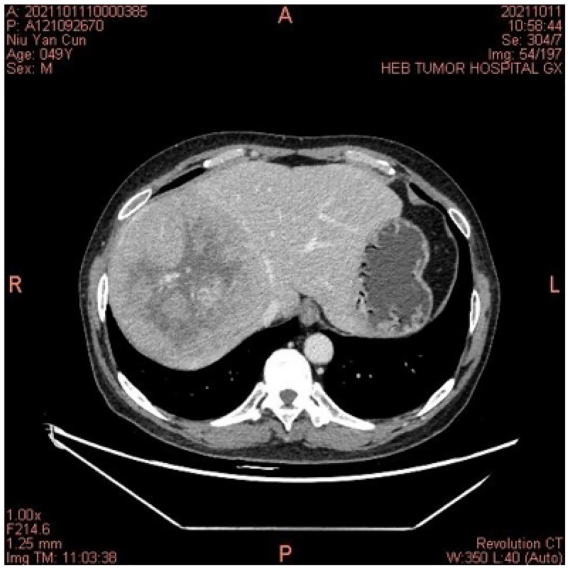

FIGURE 1
In October 2021, the computed tomography of the abdomen indicated a space-occupying lesion in the right lobe of the liver, and liver cancer was considered.

years prior and is currently taking aspirin, ezetimibe tablets, and rosuvastatin regularly. He had no prior history of infectious diseases or other surgical history. With regard to family history, the patient's mother died due to esophageal cancer. The father was alive and denied any history of hepatitis-related or genetic diseases in the family. Diagnosis at admission included (i) primary liver cancer; (ii) posthepatitic cirrhosis; (iii) chronic active viral hepatitis B; (iv) coronary heart disease, coronary stent immediately after the postoperative period; and (iv) very high risk of Grade 1 hypertension. The electrocardiogram on admission showed a ventricular rate of 66 bpm, sinus rhythm, QA pattern in V1 and V2, and Q waves in III and aVF. Imaging studies revealed a huge mass, approximately 13 cm in size. Potential for surgical examination was evaluated based the patient's physical condition and residual liver volume. Considering that the patient's liver cancer lesion was located in the right liver and the volume was huge, it was considered that if surgical treatment was performed, the residual liver volume would be less than 40%. Thus, the risk of surgical treatment was high, and the patient was at increased risk of postoperative liver failure. Hepatic arterial infusion chemotherapy (HAIC) combined with tislelizumab was recommended. Baseline thyroid function tests included free triiodothyronine (FT3) 3.15 pmol/L (3.1–6.8), free thyroxine (FT4) 11.09 pmol/L (11–20), serum thyroid stimulating hormone (TSH) 4.25 uIU/mL (0.270–4.200), thyroglobulin antibody (TgAb) 307.1 IU/mL (0-115), and thyroid peroxidase antibody (TPOAB) 7.98 IU/mL (0–34). Two cycles of HAIC (oxaliplatin 100 mg + fluorouracil 3 g + levofolinate calcium 350 mg) combined with immunotherapy with tislelizumab 200 mg were administered on 18 October and 16 November, respectively. Thyroid function tests did not show any abnormalities during this period.

On 27 December 2021, the patient experienced systemic pruritus, poor appetite with trembling of the hands, irritability, and weight loss, and severe difficulty falling asleep. Physical examination did not show yellowing of the skin mucosa or sclera of the whole body, multiple scratches on the upper limbs of the trunk and skin damage, mild bulging of the eyeballs, and no maculopapule on the skin. Thyroid function tests showed FT3 20.95 pmol/L, FT4 67.17 pmol/L, serum TSH 0.01 uIU/mL, TgAb 819 IU/mL, and TPOAB 8.24 IU/mL. Thyroid ultrasound revealed a thickness of 1.8 cm in the right thyroid lobe, 0.4 cm in the isthmus, and 1.8 cm in the left lobe. The thyroid echoes were ancestral, reduced and heterogeneous, and the diagnosis indicated diffuse thyroid lesions (Figure 2). ECG revealed a ventricular rate of 144 bpm, atrial fibrillation with a rapid ventricular rate. On 28 December, examination of pituitary hormone level and the pituitary magnetic resonance scan did not show any abnormalities. A complementary diagnosis of hyperthyroidism was made based on these findings. After consultation with the endocrinologist and considering the severe adverse reaction of antitumor therapy, immunotherapy was interrupted and the patient was given loratadine tablets 10 mg per os/day, thiamazole tablets 10 mg per os 2/day, and methylprednisolone tablets 28 mg/day. CT imaging on 28 December 2021 showed a large mass shadow of mixed density in the right lobe of the liver measuring approximately 14.2x13.4 cm (Figure 3).

Thyroid function tests on 7 January 2022 revealed FT3 2.52 pmol/L, FT4 56.4 pmol/L, and serum TSH 0.006 uIU/mL. The patient had normal diet, normal sleep patterns, no special discomfort, and hyperthyroidism improved significantly. The electrocardiogram showed a ventricular rate of 61 bpm and sinus rhythm. The third cycle of HAIC combined with tislelizumab was administered on 14 January. On 12 February, thyroid function tests showed free FT3 1.67 pmol/L, free FT4 4.58 pmol/L, serum TSH

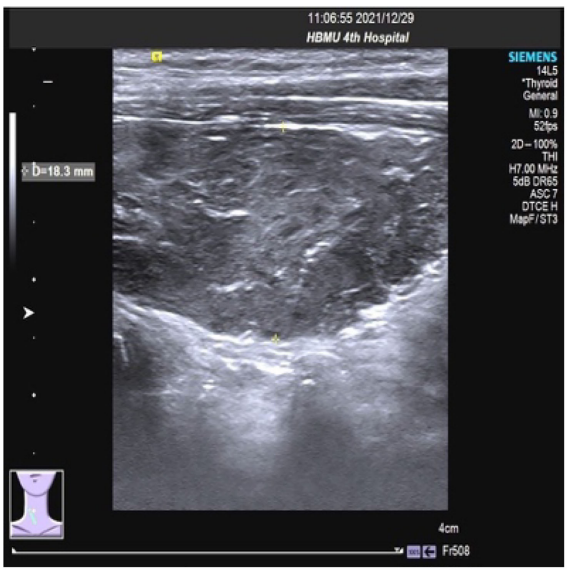

FIGURE 2
Thyroid ultrasound results showing diffuse thyroid lesions.

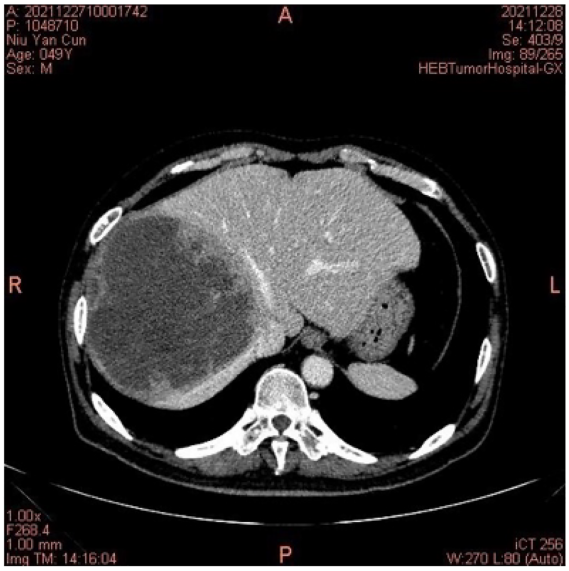

FIGURE 3
On 28 December 2021, the computed tomography of the abdomen indicated right lobe liver cancer.

54.97 uIU/mL, TGAB 408.6 IU/mL, and TPOAB 7.51 IU/mL. Physical examination revealed no facial edema, no tenderness in the neck, and no palpable enlargement of the thyroid gland. A supplementary diagnosis was made of immune-related thyroiditis, hypothyroidism. Levothyroxine sodium tablets 12.5 μg once daily were administered orally for thyroiditis. The CT performed on 14 February 2022 showed a large mixed density shadow in the right lobe of the liver that measures approximately 12.7x12.1 cm. Considering that the lesion was not significantly smaller than before, despite the general improvement, stable disease (SD) was evaluated for 4 months.

Subsequent treatment was changed to transcatheter arterial chemoembolization (TACE) combined with lenvatinib mesylate. Three cycles of TACE combined with lenvatinib mesylate were performed from 17 February 2022 to 29 April 2022, and then treatment was changed to 8 mg of lenvatinib mesylate once daily as maintenance therapy. On 27 April 2022, reexamination of thyroid function showed free FT3 2.48 pmol/L, free FT4 5.26 pmol/L, serum TSH 62.47 uIU/mL, TGAB 575.6 IU/mL, and TPOAB 8.69 IU/mL. The clinical time course of the development of thyroid disease in the present patient is shown based on several clinical parameters (FT3, FT4, TSH, TgAb, and TPOAb levels) in Table 2 and Figure 4).The patient had normal diet and physical strength, increased sleep, unresponsiveness, or other discomfort. Physical examination showed no facial edema, no tenderness in the neck, and no palpable enlargement of the thyroid gland. The dose of levothyroxine sodium tablets was adjusted to 100 μg orally once daily. Follow-up examinations on 28 April 2022, 23 July 2022, and 26 August 2022, showed a large mass in the right lobe, with no significant volume changes and no significant enhancement of injury. The complete tumor evaluation was SD for 6 months, and oral lenvatinib mesylate is being continued to date. A timeline describing diagnosis, treatment, and irAEs is shown in Figure 5.

Discussion and conclusion

Tislelizumab binds to human PD-1 with high specificity and affinity (dissociation constant, Kd 0.15 nmol/L) (21) using key epitopes GLN75, THR76, ASP77, and ARG86 present on the PD-1 receptor (11). This contrasts with nivolumab and pembrolizumab which do not require binding of these epitopes. Tislelizumab dissociates more slowly from the PD-1 receptor than nivolumab (50-fold slower) and pembrolizumab (100-fold slower). A population pharmacokinetics (popPK) model for tislelizumab showed linearity over the dose range 0.5–10 mg/kg after a single i.v. dose (12). Following a single infusion of tislelizumab 200 mg, the volume of distribution was 4.41 L and at steady state the volume was 5.247 L. Tislelizumab showed a clearance of 0.247 L/day and a half-life of 13.3 days, whereas repeated dosing in a population pharmacokinetic analysis, the clearance was 0.171 L/day and the half-life was 26 days.

Immunotherapy with ICIs uses the body's own immune system to attack cancer cells but causes unwanted autoimmune side effects in up to 60% of patients. These irAEs may lead to treatment interruption, permanent organ dysfunction, hospitalization, and premature death. Thyroiditis is one of the most common irAEs. Studies have confirmed that thyroiditis caused by ICIs is composed of T-cell-predominant but varied immune infiltrates, mainly including γδT17 cells, CD41 Th17, and CD81 Tc17 cells. The γδT17 cells are a major subset of interleukin (IL)-17A1-producing cells and early expansion may contribute to the activation and recruitment of Th17, CD81, and other T cell populations. Following treatment with ICIs, multilineage IL-17T cells expand in the thyroid tissue, and IL-17-producing innate-like γδT17 cells increase in the thyroid gland, and adaptive Th17 cells also increase significantly in the thyroid gland. Targeting Th17 and γδT17 cell function via the

TABLE 2 Clinical time course of the development of thyroid disease in the present patient is shown according to several clinical parameters.

Date	FT4 (pmol/L)	FT3 (pmol/L)	TSH (mU/mL)	TgAb (IU/mL)	TPOAB (IU/mL)
2021/10/20	11.09	3.15	4.25	307.1	7.98
2021/12/27	67.17	20.95	0.01	819	8.24
2022/1/7	56.4	2.52	0.006	556.8	–
2022/2/12	4.58	1.67	54.97	408.6	7.51
2022/3/19	5.26	2.48	62.47	575.6	8.69

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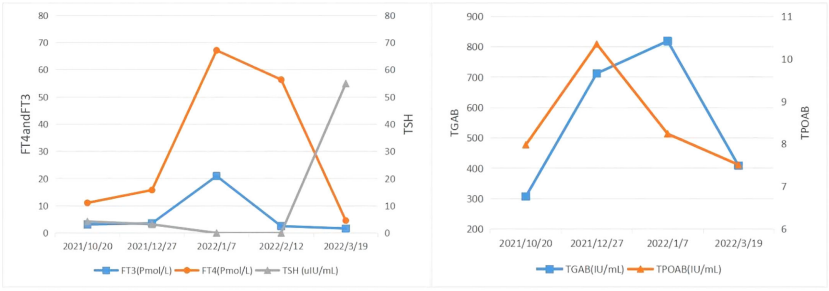

FIGURE 4
The clinical time course of the development of thyroid disease in the present patient is shown according to several clinical parameters. FT3, FT4, TSH, TgAb, and TPOAb level.

IL-17A axis may therefore be a generalizable strategy for addressing type 3 immune-mediated irAEs in the future. Several hypotheses for thyroiditis include genetic susceptibility associated with the human leukocyte antigen (HLA) haplotype associated with autoimmune thyroid disease, CTLA-4 or PD-1 polymorphisms, underlying autoimmune susceptibility, disinhibited regulatory T cell function, and/or cytokine (IL-2, interferon-α)-mediated thyroiditis (13, 14).

Endocrine dysfunction is one of the most common irAEs reported in ICI clinical trials and includes hypothyroidism, hyperthyroidism, hypophysitis, primary adrenal insufficiency (PAI), and insulin-deficient diabetes mellitus (IDD) (15, 16). ICI-induced thyroid dysfunction often occurs within weeks to months of medication, with a median onset time of 18 to 123 days, and has been reported as early as 7 days and as late as 3 years, and the median onset time is shorter in patients with hyperthyroidism than in hypothyroidism, and 80% of patients with hyperthyroidism subsequently progress to hypothyroidism, with a median time to develop this evolution of 4 to 7 weeks (17, 18).

The incidence of ICI-related thyroid dysfunction depends on two factors, one is the type of ICI and the other is the form of treatment (monotherapy or immune combination therapy). It has been reported that CTLA-4 inhibitors result in ICI-related thyroid dysfunction in 1% to 5%, and in 5% to 10% following PD-1 or PD-L1 inhibitor treatment, whereas the incidence of thyroid dysfunction is as high as 10% to 20% during immune combination therapy (19, 20). The incidences of hypothyroidism with monotherapy and with different types of ICI drug immunization combinations were further analyzed in two meta-analyses on the prevalence of endocrine disorders associated with ICIs published by de Filette et al. (20) and by Barroso-Sousa et al. (19).

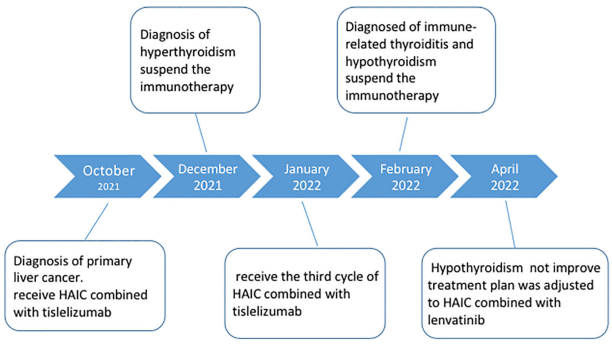

FIGURE 5
Timeline for diagnosis, treatment and immune-related adverse events.

The reported incidence of tislelizumab related thyroid dysfunction has varied widely across different clinical trials involved lung cancer, esophageal cancer, gastric/esophageal junction cancer, urothelial cancer, and classical Hodgkin's lymphoma (22–28). In the clinical trials, the incidence of hypothyroidism was 6% to 20%, the incidence of hyperthyroidism was 2.7% to 6%, and all were of Grade 1 to 2.

In our data set, there was a rare incidence of Grade 3 hypothyroidism and of hyperthyroidism events (0.2%). A retrospective analysis of the US Food and Drug Administration adverse event reporting system reported that 9.3% (171/1842) of patients presented with hyperthyroidism before hypothyroidism (29). Lee et al. found that 80% (28/35) of 35 patients with ICI-associated thyrotoxicosis eventually developed hypothyroidism (30). In addition, marked thyrotoxicosis was associated with prolonged progression-free survival (PFS) (HR 0.68, 95% CI 0.49 to 0.94; $P = 0.02$) and overall survival (OS) (HR 0.57, 95% CI 0.39 to 0.84; $P = 0.005$); however, there was no association between hypothyroidism and cancer outcomes (31). A systematic review of 47 studies showed that thyroid irAEs occurring during ICI therapy were associated with improved OS and PFS, and ICI-induced antitumor immunity and autoimmunity were associated, particularly in the setting of significant thyroid dysfunction. Other endocrine irAEs, such as hypophysitis and diabetes mellitus, are very rare, and treatment methods or disease processes themselves may decrease survival (32–34).

Potential risk factors for thyroid dysfunction caused by ICIs include the presence of antithyroid antibodies at baseline, higher TSH values at baseline, and dose, duration, sex, and higher BMI values in patients receiving anti-PD-1 antibody therapy, which may be associated with a high-risk of thyroid dysfunction (31, 35). In addition, thyroid autoantibodies may play a role in thyroid dysfunction associated with ICIs (36). Maekura et al. described 5 patients who presented ICI-associated hypothyroidism who were positive for at least 1 thyroid autoantibody, and of these, 4 (80%) were positive for both TgAb and TPOAb at baseline (37). A

retrospective study by Iwama et al. reported patients with positive baseline TgAb or TPOAb before immunotherapy had a higher incidence of thyroid dysfunction than patients with negative baseline TgAb or TPOAb levels (38). A prospective cohort study demonstrated that patients who were positive for TgAb or TPOAb were more likely to develop thyroid dysfunction after PD-1 inhibitor therapy (39). TgAb and TPOAb are risk factors for thyroid dysfunction induced by ICIs. The mechanism may be related to the fact that ICIs inhibit PD-1 signaling in follicular helper T cells, promote follicular helper T cell proliferation, increase TgAb and TPOAb levels, induce thyroid immune function loss, and thus influence thyroid function. Pollack et al. reported an increased risk of thyroid dysfunction in patients treated with PD-1 inhibitor with higher baseline TSH levels ($P = 0.05$) and a significantly higher risk of TD in patients with baseline TSH levels >2.19 mIU/mL (OR = 3.46, 95% CI 1.2-9.8) (40).

Another clinical study reported that thyrotrophin receptor antibodies (TRAbs) tested in 6 of 7 cases of irAE-associated TD, were negative, which makes Graves' disease caused by thyroid autoantibody TRAbs unlikely to be the main cause of irAEs-associated TD (41).

Multivariate analysis showed that disease duration (≥1a), thyroid color ultrasound revealing no nodules, and female sex were independent risk factors for the development of thyroid dysfunction, whereas patients with characteristics of female sex, disease duration (≥1 a), thyroid color ultrasound revealing no nodules, and elevated BMI were more likely to develop thyroid dysfunction (42–44). Drug dose may also be another important risk factor for thyroid injury caused by ICIs. The onset of irAEs related TD appears to be closely related to the dose administered and usually occurs after 2 to 4 infusions (45). Thus, baseline screening for thyroid function before initiating ICIs is particularly important, and patients with high thyroid antibodies before initial treatment are likely to develop thyroid dysfunction.

Our patient presented with hyperthyroidism during treatment cycle 3 (Day 65) of HAIC combined with tislelizumab for advanced hepatocellular carcinoma, which progressed to hypothyroidism during Cycle 5. Tislelizumab is a PD-1 inhibitor that predisposes to thyroid dysfunction. At present, there has been no relevant report describing thyroid dysfunction associated with HAIC treatment. In addition, our patient had no previous history of underlying thyroid disease, only thyroglobulin antibody was high before treatment, and other thyroid examination indicators were normal. Therefore, the patient may have developed thyroid toxicity caused by tislelizumab. The patient was assessed as having Grade 3 thyrotoxicosis and Grade 1 hypothyroidism according to the CTCAE 5.0 evaluation criteria (46).

In terms of immune-related hyperthyroidism, Chinese Society of Clinical Oncology/National Comprehensive Cancer Network (CSCO/NCCN) guidelines indicate that ICIs can be continued in patients exhibiting hyperthyroidism of Grade s 1 to 4 and β-blockers may treat symptoms, while further assessment of Graves' disease is required if persistent thyrotoxicosis occurs (47, 48). ESMO recommends that thyrotoxicosis be treated symptomatically with propranolol or atenolol, and carbimazole should be considered if thyrotropin receptor antibody is positive. Prednisolone 0.5 mg/kg should be considered for thyroiditis with pain and gradually discontinued, and if it does not improve, ICIs should be discontinued and reconsidered after symptoms are controlled (49). The ESE clinical practice guidelines do not recommend antithyroid drug therapy (such as thiamazole or radioiodine) for most cases of hyperthyroidism unless thyrotoxicosis persists for 6–8 weeks or the patient has features of Graves' disease (i.e., ocular findings, thyroid enlargement) or is positive for thyrotropin receptor antibody (50), because thyrotoxicosis is not caused by excessive thyroid hormone synthesis, but by thyroid destruction (51). Our patient discontinued treatment with the ICI tislelizumab, and in accordance with the package insert for Grade 2 or 3 hyperthyroidism, ICIs were suspended until the adverse reaction recovered to Grade 0-1, and anti-thyroid drugs were administered as needed; for Grade 4 hyperthyroidism, the drug was permanently discontinued. It has been shown that over a 16-month period, of 13/90 and 3/13 patients receiving anti-PD1 monotherapy and or combination with ipilimumab, respectively, the hyperthyroid phase ended spontaneously in all 12 patients with thyrotoxicosis without any pharmacological intervention with antithyroid drugs, but all 16 patients with thyroid dysfunction eventually required long-term levothyroxine replacement. However, thyroid dysfunction did result in ICI treatment interruption, with the longest duration of interruption being 20 weeks (mean 2; range 0–20). Following treatment for thyroid dysfunction, all patients continued ICI immunotherapy (52). Of 657 patients treated with ICI, 43 (6.5%) developed thyrotoxicosis. During the thyrotoxicosis phase, 14 (33%) patients presented with symptoms, palpitations being the most common symptom, followed by tremor, fear of heat, weight loss, and fatigue, atrial fibrillation with rapid ventricular rate, ICI therapy was not interrupted, and conservative treatment with β-blockers was sufficient for all symptomatic patients (53). Because our patient presented with Grade 3 hyperthyroidism, immunotherapy was discontinued and thiamazole tablets 10 mg b.i.d. and methylprednisolone Tablets 28 mg q.i.d., were administered concomitantly. In accordance with the guidelines and relevant literature, since our patient developed atrial fibrillation with a rapid ventricular rate, it was recommended to that β-blockers be considered to relieve symptoms without any administering antithyroid drugs (such as thiamazole tablets), unless thyrotoxicosis lasted for 6 to 8 weeks or the patient presented features of Graves' disease (i.e., ocular manifestations, thyromegaly); thus, in such cases therapy with thiamazole tablets presents limitations.

The clinical presentation and impact of IrAE are diverse and complex, and the management of patients using ICI often requires a balance between efficacy, toxicity, and specific treatments and active multidisciplinary collaboration. Patients with mild to moderate irAEs had longer OS compared to patients without irAEs. That is, the occurrence of moderate to low grade immune-related adverse events is associated with improved survival. High-grade irAEs, on the other hand, can be life-threatening and may require discontinuation of therapy or suppression of systemic immunity, which may counteract the effects of ICIs (54). Therefore, severe thyrotoxicity caused by tislelizumab treatment may have some impact on patients with hepatocellular carcinoma. Data suggests that patients with irAEs who do not respond to treatment before onset may benefit from retreatment, and that patients who respond objectively before irAEs have similar progression-free survival and overall survival in the

retreatment and off-treatment arms, and that approximately 25-50% of patients relapse after retreatment with anti-PD-1/PD-L1 antibodies. This patient had an objective response before tislelizumab caused irAEs, and suspension of treatment may have little effect on progression-free survival and overall survival, but grade 3 irAEs may be life-threatening. Therefore, to minimize the incidence of irAEs, full consideration should be given to patient tolerability and the severity of irAEs.

Immune-related hypothyroidism is usually permanent and requires thyroid hormone replacement therapy (55). The CSCO/NCCN/ESMO guidelines recommendations are very similar: clinical observation is only required when Grade 1 hypothyroidism is asymptomatic and no treatment is required, ICIs are continued, and levothyroxine sodium tablets are used for replacement therapy when Grade 2-4 hypothyroidism continues (49, 56, 57). The CSCO guidelines emphasize that hypothyroidism and other endocrine toxicities (such as diabetes) do not require glucocorticoid therapy, but alternative hormone therapy is recommended. ICIs may be continued if only cutaneous or endocrine symptoms are present. The NCCN recommends about 1.6 μg/kg daily oral levothyroxine (dose reduction in older patients or in patients with cardiac disease), and the ESMO guidelines recommend smaller doses (0.5 to 1.5 μg/kg) for thyroid hormone supplementation. In the package insert of tislelizumab, for Grade 2 or 3 hypothyroidism, ICIs should be suspended until the adverse reaction recovers to Grade 0-1, and thyroid hormone replacement therapy should be started as needed; if acute thyroid inflammation is suspected, discontinuation of tislelizumab and corticosteroid therapy may be considered. For Grade 4 hypothyroidism, treatment with tislelizumab must be permanently discontinued. Thyroid function should be monitored to ensure appropriate hormone replacement therapy. In one study of 657 patients treated with ICI, 37 (84%) developed hypothyroidism and subsequently started thyroid hormone replacement therapy. Four (9%) patients recovered from transient hypothyroidism and did not require levothyroxine, and 2 patients died before developing hypothyroidism. The median levothyroxine dose required to achieve euthyroid status was 1.2 μg/kg (range 0.25–3 μg/kg). Patients in this study were followed for a prolonged period (>14 months) after the onset of hypothyroidism, and all patients who started levothyroxine remained on thyroid hormone replacement therapy at the last follow-up, suggesting that hypothyroidism may require lifelong treatment (53). In this patient, tislelizumab 200 mg combined with HAIC was continued for 1 cycle after significant improvement of hyperthyroidism, and the patient progressed to hypothyroidism (TSH 54.97 μIU/mL) and was supplemented with levothyroxine sodium tablets 12.5 μg/day. Thyroid function was followed up 2 months later: FT3 2.48 pmol/L, FT4 5.26 pmol/L, TSH 62.47 μIU/mL. Levothyroxine sodium tablets were dose adjusted to 100 μg q.i.d. According to the CTCAE 5.0 evaluation criteria, for treatment of hypothyroidism Grade 1, the patient was asymptomatic, and it was reasonable to continue the use of tislelizumab. Our patient had coronary heart disease and hypertension; thus, in accordance with the guidelines and the package insert of levothyroxine sodium tablets, special attention was paid at the start of thyroid hormone therapy, and a lower initial dose was selected. Therefore, it was considered advantageous to carefully use levothyroxine sodium tablets in this patient.

The patient's perspective was as follows: after 2 cycles of tislelizumab, I experienced problems including poor appetite, shaking hands, atrial fibrillation, irritability, weight loss, and difficulty falling asleep. The clinician paid great attention to me throughout the treatment and found that it may be that I had had a significant adverse repose response to tislelizumab, which had improved after providing symptomatic treatment. At present, I am pleased to know that the cause of the problem was resolved by oral treatment with levothyroxine sodium tablets. I would like to thank the clinician for making this diagnosis, which improves my perception of anti-cancer therapy.

In conclusion, we report for the first time a case of severe thyroiditis caused by tislelizumab and discuss the treatment of immune-related thyroiditis, which suggests that it is necessary to improve the clinical understanding of PD-1-inhibitor-induced thyroid dysfunction and strengthen multidisciplinary collaboration in the management of such patients, especially within 1 – 2 months of treatment initiation, to monitor thyroid-related parameters, and once a patient develops immune-related thyroiditis, the patient's condition should be comprehensively assessed and appropriate treatment should be given for symptomatic treatment and treatment if necessary to improve the patient's prognosis.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article. Written informed consent has been obtained from the participant/patient for the publication of this case report.

Author contributions

PL, JH: Conceptualization, project administration, funding acquisition, writing–review and editing. LH, CW: Writing – review and editing. HD, XS: Supervision. RZ, BS: Writing –

original draft, formal analysis. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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