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Reducing adverse effects of cancer immunotherapy

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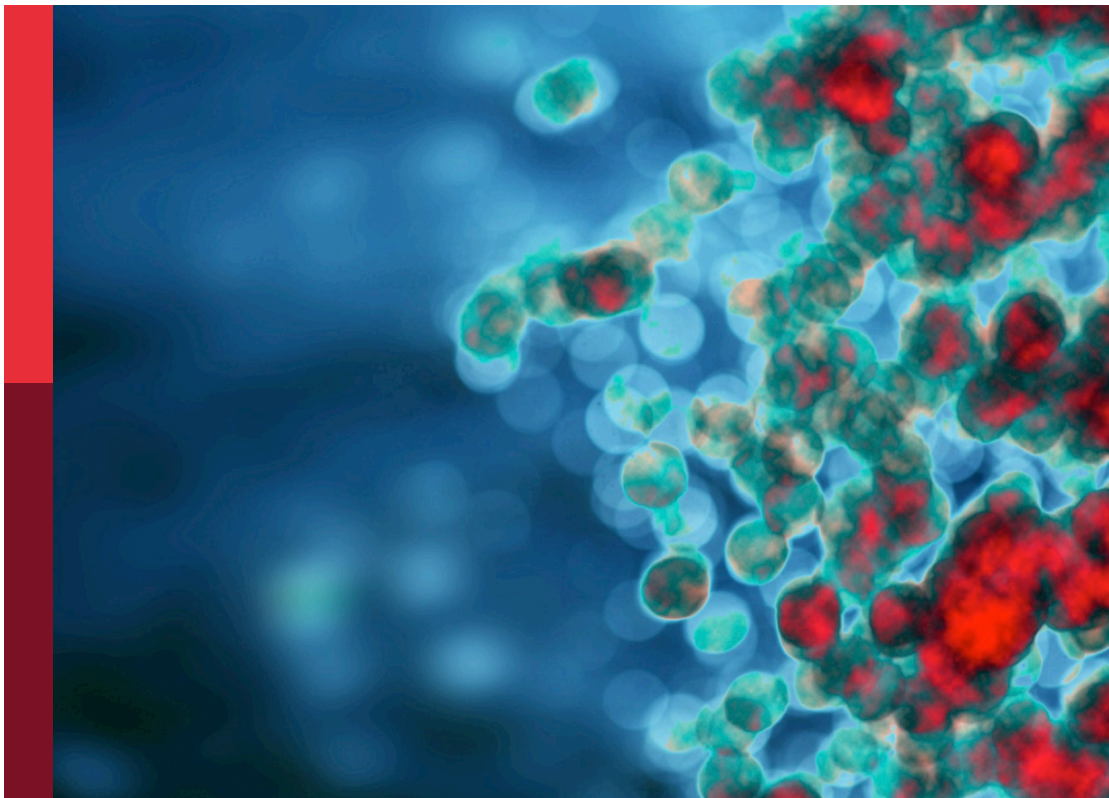

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Reducing adverse effects of cancer immunotherapy

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Table of contents

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Editorial: Reducing adverse effects of cancer immunotherapy

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Editorial on the Research Topic
Reducing adverse effects of cancer immunotherapy

Chemotherapy and radiotherapy are associated with various adverse effects. Appropriate management ensures compliance with treatment and, above all, the patient's well-being. Immunotherapy has emerged as a new and promising option for cancer treatment. However, immunotherapy is also associated with immune-related adverse events. Thirteen articles have been published on this Research Topic.

Kovalenko et al. conducted a systematic review and meta-analysis of clinical trials investigating the toxicity profile of combined ICI therapy versus ICI + VEGFi. ICIs inhibit the excessive activation of immune checkpoint signaling pathways, while VEGFi acts by interfering with vascular endothelial growth factor or by blocking the VEGF receptor. The authors showed that TRAEs occurred more frequently in the ICT + VEGFi group and treatment discontinuations were attributed to these adverse events. The largest increase in TRAE effect size was seen for rash, hypertension, hypothyroidism and diarrhea, but other TRAEs such as nausea, anorexia and anemia were also common. The results suggest that combination therapy is directly associated with a higher risk of some TRAEs compared to monotherapy.

Qiu et al. evaluated the efficacy and clinical safety of trilaciclib, a CDK4/6 inhibitor that protects blood cell lines from chemotherapy, for the prevention of chemotherapy-induced myelosuppression through a meta-analysis. Only 4 randomized controlled trials of patients with small cell lung cancer or breast cancer were evaluated. The use of trilaciclib reduced the incidence and duration of severe neutropenia, the incidence of febrile neutropenia and anemia. The therapeutic use of erythropoiesis-stimulating agents, granulocyte colony-stimulating factors and erythrocyte transfusions was also reduced in patients treated with trilaciclib. Overall survival and progression-free survival were identical in the control group and the trilaciclib group. In summary, trilaciclib has an acceptable safety profile, does not interfere with chemotherapy, and effectively reduces the incidence of myelosuppression.

Three case reports and a systematic analysis of case reports were published. Wu et al. described a case of cardiotoxicity due to the use of Tislelizumab, a PD-1 inhibitor. The case

involved a 59-year-old male patient with a history of non-small cell squamous cell carcinoma of the lung and coronary artery disease. After cardiotoxicity occurred, tislelizumab was discontinued and replaced with sugemalimab, a PD-L1 inhibitor, after which no further cardiac changes were observed. The authors assume that these results could contribute to the optimization of cancer immunotherapy.

Ohmura et al. reported a case of RS3PE after the use of nivolumab, a monoclonal antibody that targets the anti-PD1 receptor, for the treatment of gastric cancer. Despite the decrease in tumor markers and metastatic lymph node lesions, the patient showed symptoms of RS3PE syndrome, such as pain, edema, lymphocytic and macrophage infiltration in skin, and CD4+ or CD8+ T-cell infiltration in the perivascular area. Therapy with prednisolone, which inhibits inflammatory cells and suppresses the expression of inflammatory mediators by binding to glucocorticoid receptors, was initiated, and the patient was referred to supportive care. The authors suggest that the results will be useful to elucidate immune-related adverse events triggered by anti-PD-1 drugs.

Zhou et al. describes a case of tislelizumab-induced uretitis/cystitis in a male patient with thymic carcinoma. The patient was treated with tislelizumab, a humanized IgG4 monoclonal antibody with a strong affinity for PD-1 binding; paclitaxel-albumin, an antimicrotubule combined with albumin; and carboplatin, a second-generation platinum (II) complex that acts as an atypical alkylator, binds and cross-links the DNA, leading to breakage of the DNA strand during replication. The patient was hospitalized after suffering from severe abdominal and back pain that was not relieved by antibiotics and antispasmodics. The patient had dilation of the urinary tract and cystitis, which resolved after discontinuation of tislelizumab. The authors compare their case report with other cases of cystitis associated with immunotherapy and re-emphasize that the data presented may contribute to the diagnosis of unique immune-related adverse events.

Finally, Wang et al. performed a systematic analysis of case reports to evaluate evidence of ICI-associated myocarditis. A total of 113 publications from 106 patients were analyzed, with myocarditis occurring in 53.8% of cases and more than half of the cases being fatal or severe. The authors concluded that treatment of high-grade myocarditis associated with ICI use should be managed with strategies that include, for example, discontinuation of ICIs in conjunction with high-dose glucocorticoids. The information provided by the authors may assist in medical decision making.

Yang et al. investigated the possible association between aspirin, a non-steroid anti-inflammatory drug, use and irAEs in patients receiving immunotherapy. Information from the FAERS was used for this purpose. An association between aspirin use and an increased risk of irAEs was found in patients with lung cancer, mesothelioma, and pancreatic cancer, while there was a lower risk in patients with lymphoma. Major irAEs included anemia, myositis, colitis and others. Aspirin use was associated with a lower risk of skin rash, thyroiditis, and Stevens-Johnson syndrome. This information may be useful for future studies on individualized treatment plans.

In a retrospective study, Wang et al. investigated the effect and safety of prednisone, a steroidal anti-inflammatory drug, on persistent hematologic toxicity after CAR-T cell therapy, that is suggested to induce a T-cell response against antigen-expressing cells, in 17 patients with acute B-cell lymphoblastic leukemia. Administration of prednisone at an initial dose of 0.5 mg/kg/day resulted in 100% recovery of blood counts and a complete recovery of 60 to 66.67%. Hematologic toxicities recurred in 6 patients after discontinuation of treatment. The median follow-up time was approximately 14 months, progression-free survival was 58.8% and overall survival was 64.7%. The authors suggest that treatment with prednisone could be an interesting option for hematologic toxicity due to treatment with CAR-T cells.

Li et al. retrospectively analyzed 12 medical records of patients with interstitial lung disease that occurred after taking antineoplastic drugs. The authors showed that DILD was triggered by different classes of drugs, with the use of ICIs accounting for approximately 66% of cases. The authors pointed out that DILD occurs mainly in male, elderly patients with lung cancer and that some specific measures and special care are needed to improve the prognosis of DILD.

Gutierrez et al. retrospectively investigated the effects of anti-CD20 maintenance on both responses to the SARS-CoV-2 vaccine and the incidence/severity of COVID-19. The monoclonal anti-CD20 antibodies can act via several mechanisms. A significantly increased risk of severe COVID-19 within the first 24 months after the last administration of anti-CD20 was observed. Neither vaccine response nor hypogammaglobulinemia had a significant impact on overall survival. The results suggest that anti-CD20 therapy impairs the serologic response to SARS-CoV-2 vaccines. However, certain measures, such as monitoring the intake of immunoglobulins or ensuring adequate immunization, may help to mitigate this effect.

In contrast to other studies, Gong et al. used a sophisticated machine learning approach to develop a model to predict individual risk of ICI-induced IRP. Retrospective data from 48 patients with IRP and 142 without IRP who were treated with ICIs were included. Eleven predictors were used, including history of lung disease and cancer stage. The model validation showed good discrimination and acceptable calibration ability, with AUC values of 0.81, an average precision of 0.76, a scaled Brier score of 0.31, and a Spiegelhalter z of −0.29. An online risk calculator was developed, and the authors concluded that the prediction model is accurate and can be used in clinical practice.

Huang et al. also used a machine learning approach, but to identify risk factors that contribute to the development of CIM in patients with gastrointestinal cancer. Frequency and severity of CIM were analyzed in 328 patients. The authors showed significant correlations between the incidence of mucositis and gender, the number of chemotherapy cycles and the administration of platinum-based drugs, that cross-link DNA strands and inhibit DNA synthesis and function, and irinotecan, that inhibits the action of topoisomerase I by interfering with the moving replication fork and causing replication arrest and lethal double-strand breaks in DNA. A positive correlation between the occurrence of diarrhea and surgical history, treatment with irinotecan and the use of

probiotics (p = 0.037, 0.021 and 0.035, respectively) and a negative correlation with platinum-based treatment (p = 0.026) were found. Thus, the authors have successfully completed the development and implementation of the prediction model.

Park et al. used HUVECs cells and male C57BL/6 mice to test the ability of CU06-1004, a blocker of endothelial dysfunction, to inhibit endothelial permeability induced by HDIL-2, a recombinant form of human IL-2 that binds to the IL-2 receptor and activates various signaling pathways. Treatment with CU06-1004 promoted the maintenance of cellular stability and prevented HDIL-2-induced vascular leakage *in vitro* and *in vivo*, respectively. Co-administration of HDIL-2 and CU06-1004 effectively reduced tumor growth in the B16F10 mouse model. In conclusion, the authors emphasize that CU06-1004 prevents vascular leakage syndrome and has anti-cancer potential.

The treatment of cancer is still accompanied by various toxic off-target effects. Further research is essential to improve understanding and ensure optimal treatment with minimal discomfort for patients. This Research Topic has provided new and pertinent information that will make a valuable contribution to the advancement of knowledge in this field. We would like to thank all the authors, reviewers and editors who have contributed to this Research Topic.

Author contributions

DM-F: Conceptualization, Writing – original draft, Writing – review & editing. EF: Conceptualization, Writing – original draft, Writing – review & editing. CMS: Conceptualization, Writing – original draft, Writing – review & editing. CSS: Writing – original draft. AM: Writing – original draft. LB: Writing – original draft.

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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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Low-dose administration of prednisone has a good effect on the treatment of prolonged hematologic toxicity post-CD19 CAR-T cell therapy

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Introduction: Hematologic toxicity (HT) is a joint adverse event after CAR-T cells infusion. Some patients experience prolonged hematologic toxicity (PHT), which is challenging to treat.

Methods: We collected clinical data from patients with relapsed refractory B-ALL treated with CD19 CAR-T cells. Patients with PHT who did not respond to erythropoietin, platelet receptor agonists, transfusion, or G-CSF and eventually received low-dose prednisone therapy were included in the analysis. We retrospectively analyzed the efficacy and safety of low-dose prednisone on PHT.

Results: Among 109 patients treated with CD19 CAR-T cells, 78.9% (86/109) of patients were evaluated as PHT. Of these, 15 patients had persistent hematological toxicity after infusion (12 were grade 3/4 cytopenia, 12 were trilineage cytopenia and 3 were bilineage cytopenia), 2 developed cytopenia without apparent cause after D28. The initial prednisone dose was 0.5 mg/kg/day, and the median response time was 21 days (7-40 days). The recovery rate of blood count was 100%, and the complete recovery rate ranged from 60% to 66.67%. Especially exciting was that HT recurred in 6 patients after stopping prednisone. They were relieved again after the administration of prednisone. The median follow-up time was 14.97 months (4.1-31.2 months). Twelve-month duration of PFS and OS rates were 58.8% (±11.9%) and 64.7% (±11.6%). We did not observe any other side effects of prednisone apart from drug-controllable hyperglycemia and hypertension.

Discussion: We suggest that low-dose prednisone is a beneficial and tolerable therapy for PHT after CAR-T cells. The trials have been registered at www.chictr.org.cn as ChiCTR-ONN-16009862 (November 14, 2016) and ChiCTR1800015164 (March 11, 2018).

KEYWORDS

CAR-T cells, acute lymphocytic leukemia, prolonged hematologic toxicity, prednisone, hematopoietic recovery

Introduction

The efficacy of chimeric antigen receptor T (CAR-T) T cells for treating hematologic malignancies has been widely recognized. CD19 CAR-T cells have achieved complete remission (CR) rates of over 90% for B-cell acute lymphocytic leukemia (B-ALL) (1). The usual adverse events after CAR-T cells are cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), with an incidence of approximately 81% and 40%, respectively (2). However, little attention has focused on hematologic toxicity (HT), with the incidence as high as 90% (3–7). In general, approximately 63.3% of cytopenia patients had restored normal blood count at 13 months after transfusion (8, 9), and 16% of patients still had hemocytopenia even at the follow-up of 22 months (10). Prolonged hematologic toxicity (PHT) increases the incidence of infection, bleeding, and fatigue in patients, which should be taken seriously.

Higher tumor burden, multiple lines of prior therapy, and pretreatment with lymphodepletion may impair bone marrow hematopoiesis (11, 12), which may contribute to the occurrence of PHT after CAR-T cell therapy. Furthermore, some recent studies showed that subsequent malignancies, such as myelodysplastic syndrome (MDS) and clonal hematopoiesis of indeterminate potential (CHIP) (8, 10, 13), occurred in a proportion of PHT patients. However, these factors do not fully explain the occurrence of PHT. PHT may be related to bone marrow suppression triggered by CAR-T cells, increased inflammatory factors, or the activation of excessive immune responses (14).

Glucocorticoids are commonly used to manage high-grade CRS and ICANS. However, the effect of glucocorticoids on PHT is uncertain. In this study, we enrolled 17 patients with relapsed/refractory B-ALL (R/R B-ALL) who developed PHT after CAR-T cell therapy and evaluated the effect and safety of low-dose prednisone on long-term hematologic recovery.

Methods

Patients and data collection

We retrospectively analyzed R/R B-ALL patients treated with CD19 CAR-T cells from September 2019 and September 2022. All enrolled patients participated in a single-center clinical trial of CAR-T cell therapy targeting CD19 (ChiCTR-ONN-16009862 and ChiCTR1800015164). The inclusion criteria were as follows: 1) diagnosis of R/R B-ALL, 2) treatment with CD19 CAR-T cells, 3) CR at D28, 4) HT remained in D28, and 5) receiving low-dose prednisone therapy. The exclusion or termination of follow-up criteria were as follows: 1) bridging to hematopoietic stem cell transplantation (HSCT) or other radiotherapy or chemotherapy regimens after CAR-T cell treatment; 2) secondary to or combining with hematopoietic disorders such as MDS or CHIP; and 3) receiving other treatments or drugs that interfere with the efficacy of prednisone during oral prednisone administration, such as granulocyte colony-stimulating factor (G-CSF), erythropoietin, platelet receptor agonists.

The primary objective was to assess the efficacy and safety of low-dose prednisone in PHT that was ineffective for G-CSF or blood transfusion after CAR-T treatment. The secondary objective was to assess the effect of prednisone on CAR-T cell efficacy.

The study was approved by the institutional review board at Tianjin First Center Hospital and was conducted according to the Good Clinical Practice guidelines of the International Conference on Harmonization. The patients were informed about the treatment regimen's potential clinical benefits and adverse events (AEs), including CD19 CAR-T cells and glucocorticoids, and provided written informed consent. The Tianjin First Central Hospital Medical Ethics Committee granted ethical approval for this study.

CD19 CAR-T cell infusion

CD3 T cells were isolated from peripheral blood mononuclear cells of patients or healthy donors by using CD3 immunomagnetic beads and then cultured with a medium containing CD3/CD28 stimulating beads. The lentiviral vector containing CD19-28ζ CAR was then transduced into these cells. Finally, CAR-T cells would expand to a sufficient number for infusion. The transfection efficiency of CD19 CAR-T cells was approximately 50%. Patients would receive cyclophosphamide 300 mg/m^2 and fludarabine 30 mg/m^2 daily for 3 to 2 days prior to CAR-T cell infusion, and they were treated with CD19 CAR-T cells on D0. CRS was prospectively graded using the Lee scale (with initial patients retrospectively graded) (15).

Definition, treatment, and recovery criteria of HT

Patients with anemia, thrombocytopenia, and/or neutropenia on D28 were considered to be experiencing PHT. The criteria for cytopenia were defined according to the Management of Immune-Related Adverse Events in Patients Treated With Chimeric Antigen Receptor T-Cell Therapy: ASCO Guideline (16). The specific standards are shown in Table 1.

Patients who had not responded to G-CSF, blood transfusions, or other similar therapies began to take low-dose prednisone 1 month after CAR-T cell infusion. The initial dose of prednisone was 0.5 mg/kg/day, which was halved after the blood count recovered a grade and gradually reduced until it was discontinued during hematologic recovery. The patients used calcium tablets, gastric mucosa protectors, and anti-fungal drugs to prevent the side effects of prednisone.

We defined hematologic recovery as the absence of blood transfusion and G-CSF support. Recovery was considered when

Abbreviations: HT, hematologic toxicity; PHT, prolonged hematologic toxicity; CAR-T, chimeric antigen receptor T; CR, complete remission; B-ALL, B-cell acute lymphocytic leukemia; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; MDS, myelodysplastic syndrome; CHIP, clonal hematopoiesis of indeterminate potential; R/R, relapsed/refractory; HSCT, hematopoietic stem cell transplantation; AEs, adverse events; G-CSF, granulocyte colony-stimulating factor; OS, overall survival; PFS, progression-free survival time.

TABLE 1 The criteria for cytopenia.

Grading	Anemia (g/L)	Thrombocytopenia ($\times10^9$/L)	Neutropenia ($\times10^9$/L)
G1	LLN–10.0	>75	>1.5
G2	<10.0–8.0	>50	>1
G3	<8.0	>25	>0.5
G4	Life-threatening	<25	<0.5

G, grade; LLN, lower limit of normal.

hematocrit recovered to G2, and complete recovery was considered when it returned to standard levels. We defined the median response time as the period from the start of oral prednisolone to the recovery of at least one-degree anemia, thrombocytopenia, or neutropenia.

Statistical analysis

The results regarding patient characteristics were obtained using descriptive statistics. Overall survival (OS) and progression-free survival time (PFS) were calculated by the Kaplan–Meier analysis. OS was defined as the time from CAR-T cell treatment to death. PFS was defined as the time from CAR-T cell treatment to disease progression or death. Statistical analyses were performed using the SPSS v19.0 software (Chicago, IL, USA) and the GraphPad Prism v9 software (GraphPad, La Jolla, CA, USA).

Results

Essential characteristics of patients enrolled

Among 159 patients treated with CD19 CAR-T cells, 109 had evaluated blood count follow-up data, and 78.9% (86/109) of patients were evaluated as experiencing PHT. Fifteen patients did not respond to erythropoietin, platelet receptor agonists, or blood transfusion and G-CSF dependence. Two developed cytopenia without apparent cause after D28. Our analysis included them eventually for treatment with oral low-dose prednisone (Table 2); 52.94% (9/17) were male, and the median age was 31 years (range 8–66). The detailed characteristics of the patients are shown in Table 3. Except for one patient with the acute lymphoblastic transformation of chronic lymphocytic leukemia, the other patients had B-ALL; 47.06% (8/17) of patients had extramedullary disease, and 29.41% (5/17) had central nervous system infiltration. The median number of therapy lines before CAR-T cell infusion was 5 (range 2–15).

Efficacy and adverse events of CAR-T cells

The median follow-up time was 14.97 months (4.1–31.2 months). Twelve-month PFS and OS rates were 58.8% (± 11.9%) and 64.7% (± 11.6%), respectively. All patients had CRS. The incidence of grade 1/2 CRS was 88% (15/17), the incidence of grade 3 CRS was 12% (2/17), and no patients suffered neurotoxicity. CRS symptoms disappeared after symptomatic treatment.

Within 28 days after CAR-T cell infusion, 96.12% (16/17) of patients had anemia, 88.24% (15/17) had thrombocytopenia, and 100% (17/17) had neutropenia (Figure 1A). The proportion of patients with trilineage cytopenia was 82.35% (Figure 1B). A majority of these events were grade 3–4 cytopenia, including 41.18% (7/17) of grade 3–4 anemia, 64.71% (11/17) of grade 3– thrombocytopenia, and 70.59% (12/17) of grade 3–4 neutropenia (Figure 1C).

Of the patients, 52.94% (9/17), 70.59% (12/17), and 82.35% (14/17) received hematopoietic treatment of blood transfusion, G-CSF, and drugs, such as erythropoietin and thrombopoietin receptor agonists, respectively. However, only 11.76% (2/17) of patients (P1 and P2) had complete hematologic recovery at D28; 88.24% (15/17) of patients still had varying degrees of cytopenia, defined as experiencing PHT; 80% (12/15) of patients had trilineage cytopenia, and 20% (3/15) of patients had bilineage cytopenia (Figures 1D, E). A total of 12 patients had grade 3/4 cytopenia, 26.67% (4/15) had grade 3/4 anemia, 66.67% (10/15) had grade 3/4 thrombocytopenia, and 53.33% (8/15) had grade 3/4 neutropenia (Figure 1F).

TABLE 2 Patient demographics.

Patient demographics	Total median (n = 109)	PHT group (n = 86)	Non-PHT group (n = 23)
Age, median (range), years	34 (9–68)	33 (11–68)	43 (9–66)
Sex			
Male (%)	57 (53.27)	46 (53.49)	11 (47.83)
Female (100%)	50 (46.73)	40 (46.51)	12 (52.17)
Extramedullary infiltration			
CNS (%)	10 (9.17)	9 (10.47)	1 (4.35)
Others (%)	14 (12.84)	13 (15.12)	1 (4.35)
Previous treatments			
HSCT (%)	60 (56.07)	51 (59.3)	9 (39.13)
Lines of therapy (range)	5 (0–13)	4 (1–12)	5 (0–13)

PHT, prolonged hematologic toxicity; CNS, central nervous system; HSCT, hematopoietic stem cell transplantation.

TABLE 3 Patient and disease characteristics (n = 17).

Patient	Age/ sex	Malignancy	Cytogenetics at diagnosis	Extramedullary infiltration	Lines of therapy	Prior HSCT	Blood count			CAR-T cells infused ($\times10^6$/kg)	Response	CRS	ICANS
							HGB (g/L)	PLT ($\times10^9$/L)	NE ($\times10^9$/L)				
1	8/M	B-ALL TELAML1	Untested	Yes	3	No	124	75	1.59	5.1	CR	1	0
2	40/F	B-ALL Ph+	Normal	No	7	No	86	111	0.78	1.5	CR	3	0
3	32/M	B-ALL* JAK2-E890K	Untested	Yes	5	Yes	86	44	0.95	7.65	CR	1	0
4	50/M	B-ALL Ph+	Normal	Yes	2	No	104	370	12.18	3.37	CR	2	0
5	18/F	B-ALL	Normal	No	4	Yes	114	77	5.01	1.5	CR	1	0
6	19/M	B-ALL WT1, NOTCH1	Normal	Yes	4	Yes	126	63	4.76	2.5	CR	1	0
7	34/F	B-ALL	Complex	No	4	Yes	114	77	5.01	0.3	CR	1	0
8	48/M	B-ALL MLL-AF4	Complex	No	5	Yes	118	79	0.86	4.1	CR	1	0
9	14/F	B-ALL TP53	Complex	Yes	15	Yes	88	63	1.91	1	CR	1	0
10	46/F	B-ALL	Complex	No	4	No	116	248	3.08	0.6	CR	1	0
11	20/M	B-ALL	Untested	No	5	Yes	90	74	1.52	2	CR	1	0
12	40/M	B-ALL	Complex	Yes	9	Yes	121	109	4.85	2	CR	1	0
13	22/F	B-ALL	Normal	No	9	Yes	130	127	2.77	2.79	CR	1	0
14	29/F	B-ALL RNUX1, IKZF1	Complex	Yes	4	Yes	116	143	2.33	1	CR	1	0
15	31/F	B-ALL WT1, E2A-HLF	Normal	No	7	Yes	78	59	3.79	4	CR	2	0
16	66/M	B-ALL	Complex	No	4	No	115	279	3.5	1	CR	3	0
17	20/M	B-ALL WT1	Complex	Yes	11	Yes	99	55	0.82	2	CR	2	0

M, male; F, female; B-ALL, B-cell acute lymphocytic leukemia; HSCT, hematopoietic stem cell transplantation; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; CR, complete remission.
*Acute lymphoblastic transformation of chronic lymphocytic leukemia.

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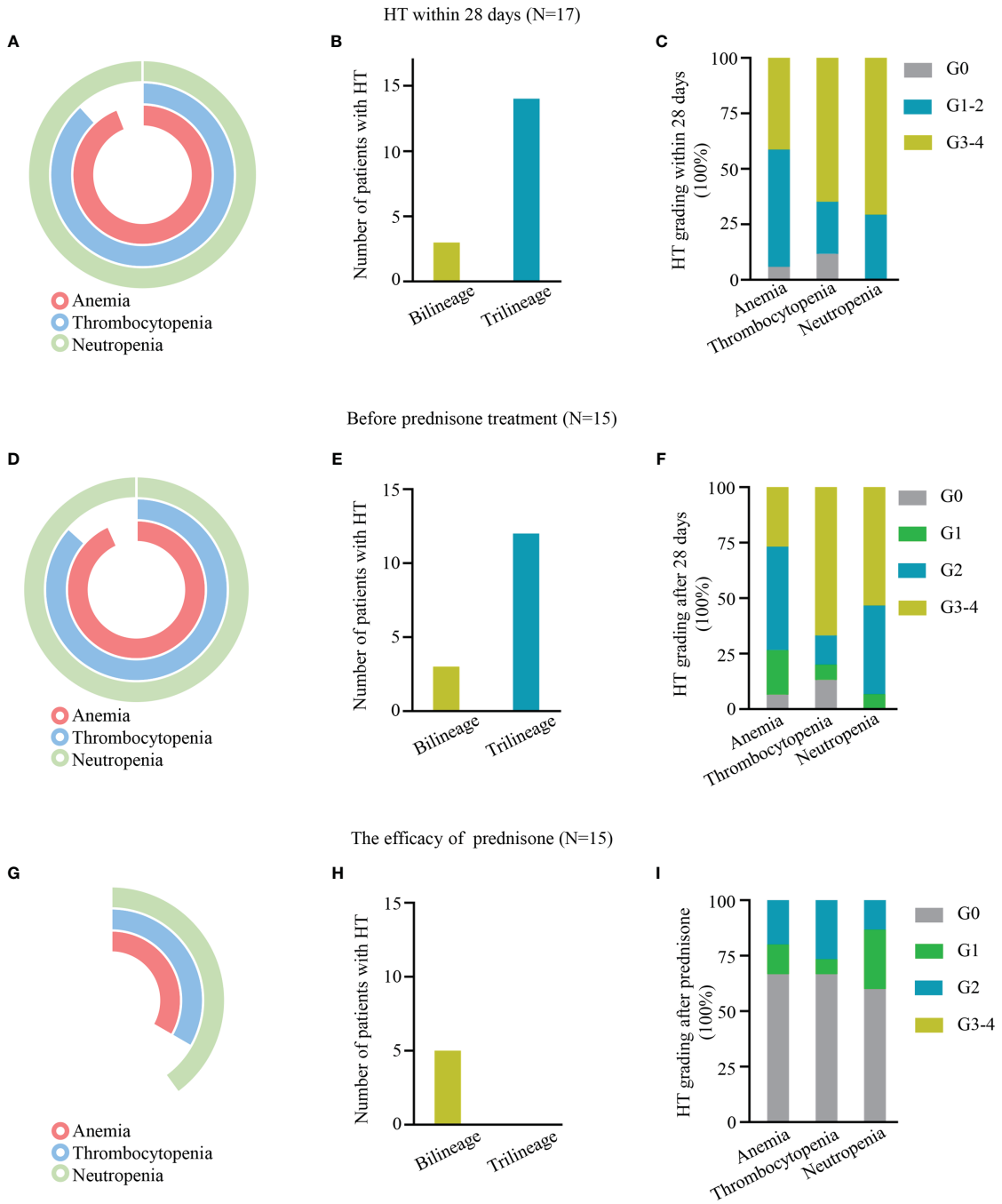

FIGURE 1
(A–C, E–G, H–I) Hematologic toxicity (HT) within 28 days, after 28 days, and after prednisone treatment, respectively, after chimeric antigen receptor T (CAR-T) cell treatment. **(A, D, G)** Pie charts depicting the incidence of anemia, thrombocytopenia, and neutropenia. **(B, E, H)** The number of HT in bilinear and trilineage response cytopenia. **(C, F, I)** The incidence of different levels of HT.

Low dose of prednisolone is effective for PHT

The median duration between CAR-T cell therapy and prednisone was 30 days (range 26–42). The median response time was 21 days (7–40 days), and all patients' blood count returned to safe levels (above G2) after prednisone treatment. Their hemoglobin count returned to standard levels in 66.67% (10/15) of patients, platelet in 66.67% (10/15), and neutrophil in 60% (9/15). Only 33.33% (5/15) of patients had bilineage cytopenia (Figures 1G–I).

Low dose of prednisone therapy was effective in patients with repeated PHT

The blood count of P1 and P2 had returned to normal levels after symptomatic treatment at D28. However, they developed

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unexplained cytopenia again around D70. They all maintained CR (Figures 2A, B). They were treated with prednisone, and their blood count returned to normal after 2 weeks of administration (Figures 2C, D).

Most interestingly, during the follow-up, we found that 40% (6/15) of patients developed HT again after stopping prednisone therapy and were effectively treated with prednisone therapy again (Figures 3A–C). P3 experienced grade 1 CRS, which disappeared soon after symptomatic management. During follow-up, P3 was in sustained remission (Figure 3D) but had grade 3/4 cytopenia. Therefore, he was started on oral prednisone on D30. His blood cell count recovered after treatment with prednisone, decreased again with discontinuation, and recovered again after administration. His blood cells fluctuated with the administration and withdrawal of prednisone (Figure 3E).

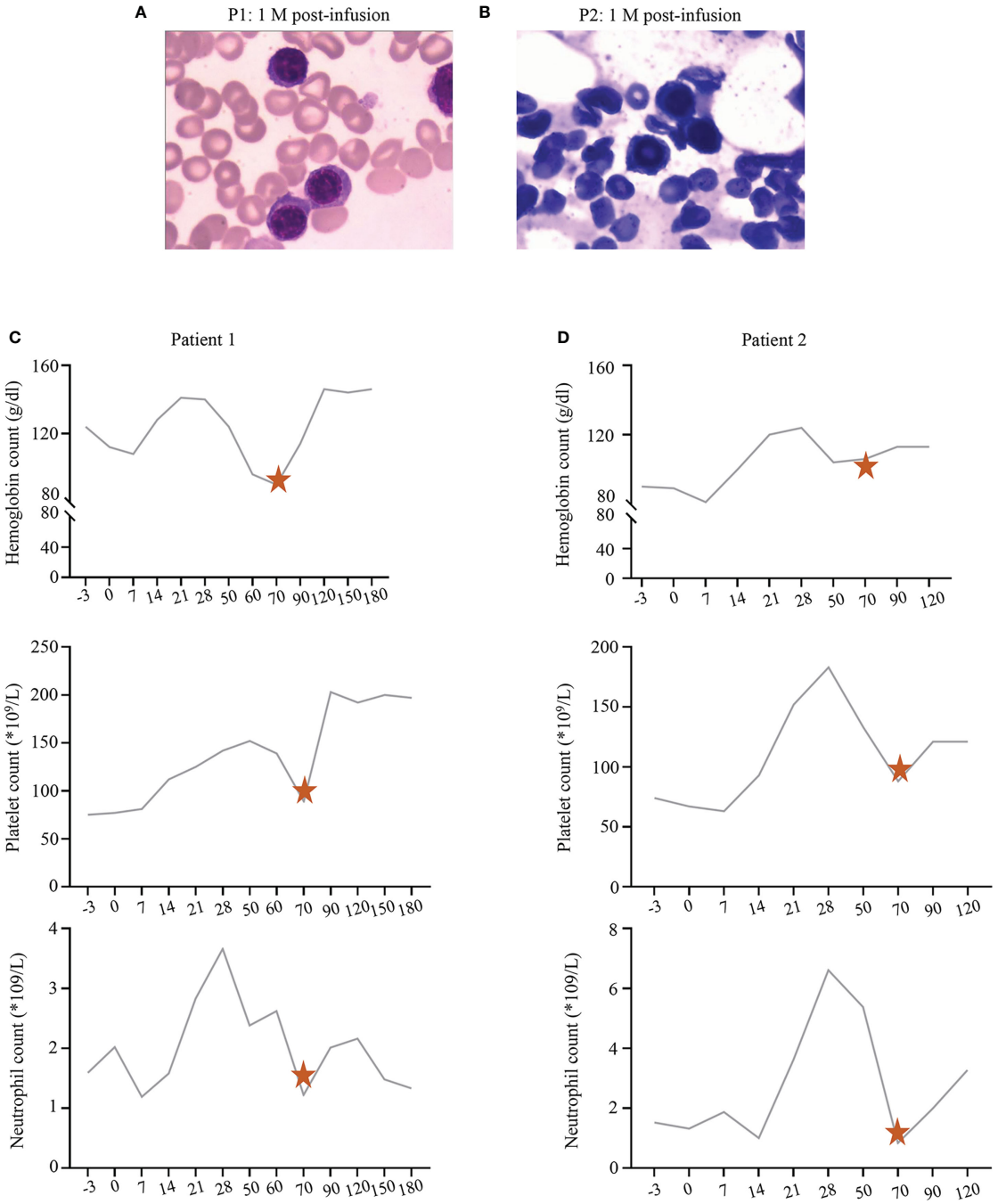

FIGURE 2
(A, B) The bone marrow status of P1 and P2 at 1 month post-infusion, respectively. **(C, D)** The recovery trend of hemoglobin, platelet, and neutrophil count in P1 and P2, respectively. The pentagrams represent the timing of prednisone administration.

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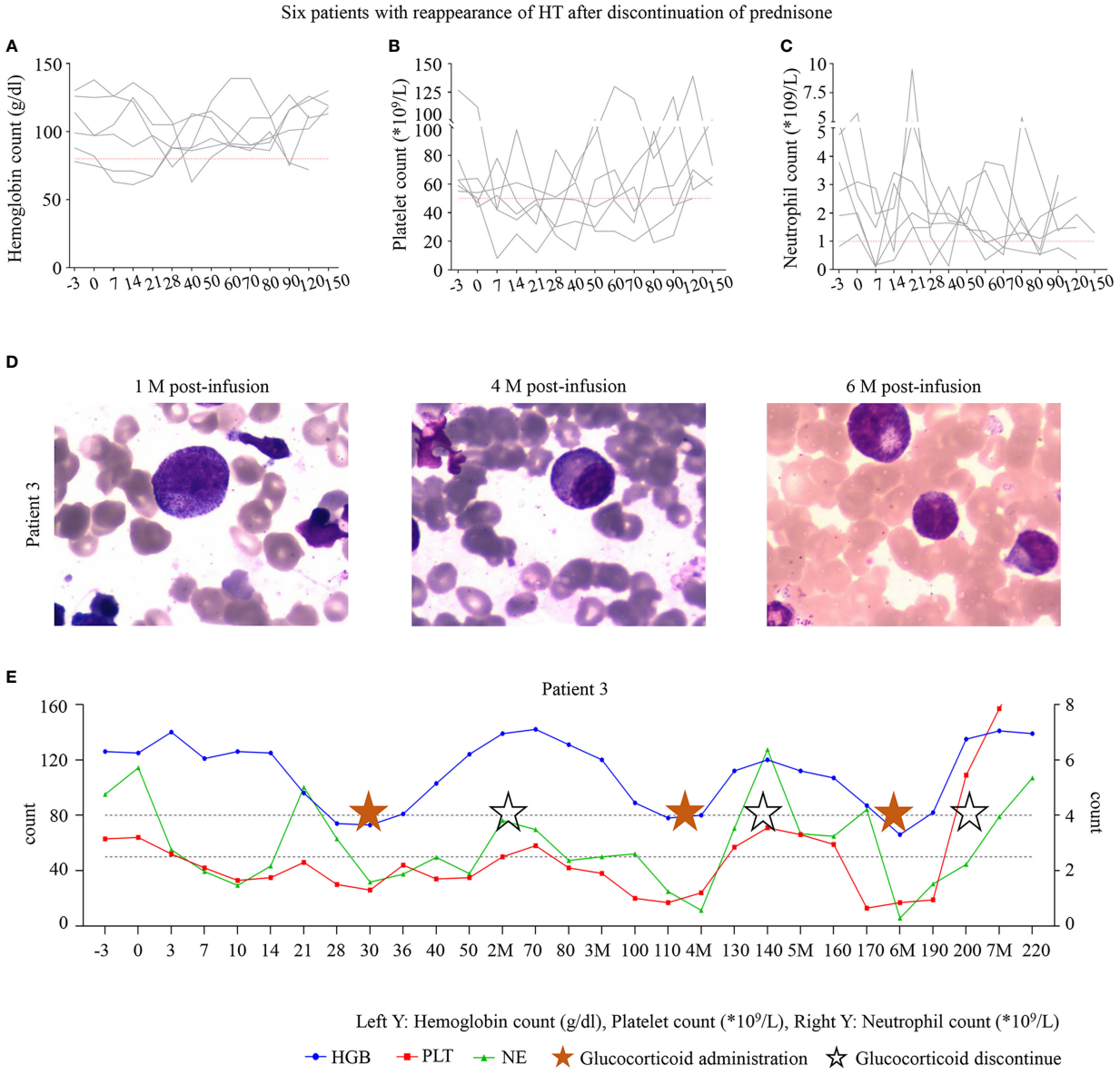

FIGURE 3
(A–C) The six patients with hematologic toxicity (HT) recurrence after discontinuation of prednisone. **(D)** The bone marrow of P3 at 1, 4, and 6 months after infusion. **(E)** The trend of blood cell count recovery in P3. P3 was administered with oral prednisone on D30 after infusion, and treatment was discontinued after hematologic recovery after 35 days of the administration. However, HT recurred after discontinuation. All three lineages were reduced to levels below grade 3 at month 4; prednisone was administered, and the patient recovered after 20 days. Trilineage reduction reappeared in month 6 after infusion and was alleviated again after prednisone administration. P3 is still undergoing follow-up. The time points marked with pentagrams are the duration of oral prednisone, and the hollow pentagrams represent the discontinuation of the drug.

Prednisolone is safe for PHT

No patients developed osteoporosis or gastrointestinal ulcers; 64.71% (11/17) of patients had hyperglycemia, and 29.41% (5/17) had hypertension, which recovered owing to oral hypoglycemic agents and antihypertensive drugs. Furthermore, after stopping prednisone treatment, the patients' blood glucose and blood pressure were not abnormal. No patient developed infections during treatment with oral prednisone.

The effect of glucocorticoids on CAR-T cell expansion and efficacy is controversial. In our study, the peak of CAR-T cell expansion in most patients was between 7 and 14 days. We found CAR-T cells detectable in 35.29% (6/17) of patients before and after prednisone treatment and remained detectable in one patient even after 305 days (Supplementary Material). Therefore, low-dose steroids may have little effect on CAR-T cells.

Discussion

The mechanism of HT is poorly understood, and the current treatment is mainly symptomatic such as transfusion of blood cells (16). We enrolled 17 patients who developed PHT after CAR-T cell therapy. Low-dose prednisone therapy promoted late hematologic

recovery in these patients. More interesting was that cytopenia developed again after stopping the therapy and recovered after steroid therapy. Therefore, low-dose prednisone may be an optional treatment option for PHT secondary to CAR-T cell therapy.

HT after CAR-T cell treatment is typical. Early hematotoxicity may be associated with different treatment options, higher tumor burden, lymphoid depletion regimens before CAR-T cell infusion, and immune and hematopoietic system destruction by CAR-T cells (9, 17–20). HT will recover after the patients achieve symptomatic treatment, such as blood cell transfusion, G-CSF, and prevention or control of infection. However, more than half of the patients experienced PHT in our study, which was consistent with previous studies (17, 21). PHT will increase the risk of infection and bleeding in patients. Nevertheless, its mechanisms still need to be further studied.

More importantly, few studies have focused on the treatment of PHT. HSCT, immunosuppressant sirolimus, and TPO receptor agonists may benefit patients with PHT (22–26). Previous studies have shown that glucocorticoids could inhibit the immune responses of T cells and B cells, reduce the production of autoantibody, alleviate antigen–antibody response, and stimulate bone marrow hematopoiesis (27). We hypothesize that glucocorticoids may promote hematologic recovery in patients with PHT. We analyzed 17 B-ALL patients with PHT secondary to CAR-T therapy to evaluate the efficacy and safety of low-dose prednisone.

We included patients with persistent cytopenia who did not respond to traditional treatments, such as growth factors and blood-promoting drugs. Excitingly, after low oral doses of prednisone, all patients' blood count recovered to safe levels (100%), with the complete recovery rate ranging from 60% to 66.67%. Interestingly, after stopping the drug, the blood cells decreased again in six patients. Their blood count recovered after continuing oral prednisone. In addition, a proportion of the patients had a biphasic pattern of HT. This pattern has been observed in previous studies (11). We found that low oral doses of prednisone were effective in patients with both sustained and bidirectional reductions.

Furthermore, during follow-up, OS and PFS were similar to those of the previous reports (28), and low-dose prednisone appeared not to affect CAR-T efficacy. We found that CAR-T cells could still be detectable after prednisone administration in six patients. This is consistent with studies that CRS glucocorticoid treatment does not affect the CAR-T cells' efficacy and proliferation (29), even under high glucocorticoid doses (30). However, our study is a single-center study with a small number of cases and a short follow-up period, so it needs to be confirmed further.

Finally, we evaluated the safety of low-dose prednisone. We found that both hypertension and hyperglycemia were reversible and improved with symptomatic treatment. Small doses of prednisone should be safe for patients with PHT.

Our results show that prednisone promotes hematologic recovery in patients with PHT. Although we do not have sufficient evidence to demonstrate the mechanism of prednisone treatment, we speculate that PHT may be associated with abnormal immune activation. Excluding the effect of other secondary diseases on PHT and monitoring immune cell subsets and immune response factors may help to understand further the mechanisms by which prednisone therapy for PHT is effective.

In conclusion, our data suggest that low-dose prednisone may improve hematologic recovery in patients with PHT after CAR-T infusion and does not sacrifice the efficacy of CAR-T cells, and the side effects are manageable. However, validation in many cases and longer follow-up are still needed.

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

The studies involving human participants were reviewed and approved by the Institutional Review Board of Tianjin First Central Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

JW and MZ wrote the manuscript draft. MFZ and WL designed the study and managed the patients. HL, XX, XB, YP, JM, QL and TY contributed to patient management. MFZ and WL were responsible for clinical trial recruitment. JW and RG collected the data, and JW and MZ analyzed the data. MFZ and WL 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 without 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.2023.1139559/full#supplementary-material

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The efficacy and safety of Trilaciclib in preventing chemotherapy-induced myelosuppression: a systematic review and meta-analysis of randomized controlled trials

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Background: This study aims to assess the clinical efficacy and safety of Trilaciclib in preventing chemotherapy-induced myelosuppression in adult patients through meta-analysis.

Methods: The PubMed, Embase, Cochrane Library, Clinical Trials, EU Clinical Trials Register, and International Clinical Trials Registry Platform were searched up to 25 October 2022. Only randomized controlled trials (RCTs) comparing the clinical outcomes of Trilaciclib and Trilaciclib plus chemotherapy for treating malignant cancers in adult patients were included. The primary outcome included the incidence of SN, FN, the DSN, and administration of ESAs, G-CSFs, and RBC or platelet transfusions, while the secondary outcomes included the risk of adverse events (AEs) and severe adverse events (SAEs).

Results: In total, four randomized controlled trials (RCTs) involving 345 patients with SCLC or breast cancer were included in this meta-analysis. Results showed that administration of Trilaciclib significantly reduced the occurrence of SN (19.3% vs. 42.2%, OR = 0.31), FN (3.22% vs. 6.72%, OR = 0.47), anemia (20.5% vs. 38.2%, OR = 0.38) and shortened the DSN during treatment. The proportion of patients receiving therapeutic use of ESAs (4.03% vs. 11.8%, OR = 0.31), G-CSF (37.0% vs. 53.5%, OR = 0.52), RBC transfusions (19.8% vs. 29.9%, OR = 0.56) was also statistically lower in the experimental group than in the control group. Meanwhile, the ORR, overall survival, and progress-free survival of the two groups were identical, and no negative impact of Trilaciclib on the clinical outcomes of chemotherapy treatments was found. Other chemotherapy-induced adverse events (AEs) and severe adverse events (SAEs) like diarrhea, fatigue, nausea, and vomiting were identical regardless of Trilaciclib usage.

Conclusion: Trilaciclib demonstrated its efficacy in reducing the occurrence of chemotherapy-induced myelosuppression and utilization of supportive care interventions without undermining the clinical benefits of chemotherapy regimens during treatment with an acceptable safety profile.

KEYWORDS
CDK4/6 inhibitor, trilaciclib, chemotherapy, myelosuppression, meta-analysis

1 Introduction

Chemotherapy is currently the cornerstone for treating many cancers like extensive-stage small cell lung cancer (SCLC), triple-negative breast cancer, etc. (Horn et al., 2018; Goldman et al., 2021; Bianchini et al., 2022). However, standard chemotherapy regimens are usually associated with myelosuppression, which may not only affect the therapeutic effect of chemotherapy but also lead to life-threatening complications like secondary infections, anemia, and bleeding. It is reported that more than 60% of patients receiving chemotherapy treatments for SCLC had at least one grade ≥3 myelosuppressive AE during treatment (Epstein et al., 2022). The incidence of chemotherapy-induced grade ≥3 neutropenia, anemia, and thrombocytopenia was 44.9%, 44.1%, and 25.4%, respectively (Epstein et al., 2022). Currently, chemotherapy-induced myelosuppression (CIM) is mainly managed with dose delay/reductions, administration of ESAs or G-CSFs, and RBC or platelet transfusions, which are burdensome to the patients and may bring other undesirable side effects (Kogan et al., 2019; Crawford et al., 2020; Epstein et al., 2020). Severe CIM affects the clinical outcome of chemotherapy treatment and imposes a financial burden on the patients and the healthcare system.

Trilaciclib is a selective and reversible inhibitor of cell cycle protein-dependent kinases 4 and 6 (CDK4/6) approved by the FDA in February 2021 as a first-in-class myeloprotective agent. Intravenous administration of Trilaciclib prior to chemotherapy can transiently arrest the CDK4/6-dependent hematopoietic stem/progenitor cells (HSPCs) and lymphocytes in the G1 phase of the cell cycle, preventing the DNA damage and apoptosis of these cells after exposure to chemotherapeutic agents (He et al., 2017). Moreover, Trilaciclib protected multilineage myeloid cells like neutrophils, red blood cells, and platelets from CIM in SCLC patients in multiple clinical trials without compromising chemotherapy efficacy and patient survival, reduced the need for supportive care interventions after treatment, improved the quality of life of the patients and provided significant clinical benefits (Dómine Gómez et al., 2021; Ferrarotto et al., 2021; Hart et al., 2021; Hussein et al., 2021). However, in another study assessing the myeloprotective effect of Trilaciclib in patients with metastatic triple-negative breast cancer, no significant differences were observed in myelosuppression endpoints between groups of Trilaciclib plus chemotherapy and chemotherapy alone, though significantly longer PFS and OS were observed (Tan et al., 2019). Moreover, some experts believed that the clinical benefits that Trilaciclib may bring to the patients should be confirmed with more extensive phase III trials and that more research was needed (Powell and Prasad, 2021). Therefore, it is necessary to systematically evaluate the preventive effect of Trilaciclib in multilineage CIM.

In this study, the CDK4/6 inhibitor Trilaciclib was investigated. Its clinical benefits and safety were compared in patients treated with therapeutic chemotherapy agents to provide a reference for clinical application.

2 Materials and methods

2.1 Study search and selection

We searched PubMed, Embase, the Cochrane Library, Clinical Trials, the EU Clinical Trials Register, and the International Clinical Trials Registry Platform (ICTRP) using "Trilaciclib" or "Cosela" or "G1T28" as search terms. ENDNOTE X 8 was used to remove the duplicate record. And after removing duplicate records from the search results, two researchers screened and reviewed each study independently. Any disagreement in the process was resolved by consulting a third researcher. All the data were extracted from the included studies, including the authorship, year of publication, study design, study duration, study site, study population, chemotherapy regimens and the comparators, clinical outcomes, and risk of AEs. The included studies should meet the following criteria: patients diagnosed with malignant cancer; age was ≥18 years old; intervention of chemotherapy, and comparison of chemotherapy vs. chemotherapy plus Trilaciclib; RCT; reporting of the efficacy outcome, including the incidence of CIM, the utilization of supportive care interventions; and the safety outcome. In this study, no ethical approval was necessary for meta-analysis in our institute.

2.2 Outcome measurement

The study's primary outcome was the rate of CIM-related AEs and the utilization of supportive care interventions. We systemically analyzed the rate of severe neutropenia (SN), febrile neutropenia (FN), the administration of erythropoiesis-stimulating agents (ESA), granulocyte colony-stimulating factors (G-CSFs), RBC or platelet transfusions, and the duration of severe neutropenia (DSN) to evaluate the protective effect of Trilaciclib from CIM. AEs like anemia, diarrhea, fatigue, leukopenia, nausea, neutropenia, thrombocytopenia, and vomiting were also statistically analyzed to evaluate the potential safety of Trilaciclib. The impact of Trilaciclib on the overall response rate (ORR), overall survival (OS), and progress-free survival (PFS) was analyzed to determine the comprehensive effect on the patients.

2.3 Data analysis

The included studies' quality and associated risk of bias were performed using the Cochrane risk-of-bias tool (Higgins et al., 2011). Two researchers subjectively reviewed all included studies and rated them "low risk," "high risk," or "unclear risk" according to the judgment items in the tool. All statistical analyses were performed by using Review Manager version 5.3. Pooled odds ratios (ORs) and 95% confidence intervals (CIs) were used to measure the association between outcomes and the use of Trilaciclib. Study heterogeneity was presented using the

TABLE 1 Characteristics of selected studies.

Study, year published	Intervention		Patient number		Study duration	Study population
	Control	Experimental	Control	Experimental		
J. M. Weiss, 2019	E/P plus placebo	E/P plus Trilaciclib	37	38	between June 2015 and February 2019	≥18 years, histologically or cytologically confirmed ES-SCLC.
Davey Daniel, 2020	Placebo prior to E/P/A	Trilaciclib prior to E/P/A	53	54	between June 2017 and February 2018	≥18 years, with confirmed ES-SCLC.
Lowell L. Hart, 2021	Placebo prior to topotecan	Trilaciclib prior to topotecan	29	32	between October 2015 and October 2021	≥18 years, with confirmed diagnosis of ES-SCLC.
Antoinette R Tan,2019	G/P plus placebo	G/P plus Trilaciclib (D1+D8)	34	33	between February 2017 and May 2018	≥18 years, recurrent or metastatic triple-negative breast cancer who had no more than two previous lines of chemotherapy
		G/P plus Trilaciclib (D2+D9)		35		

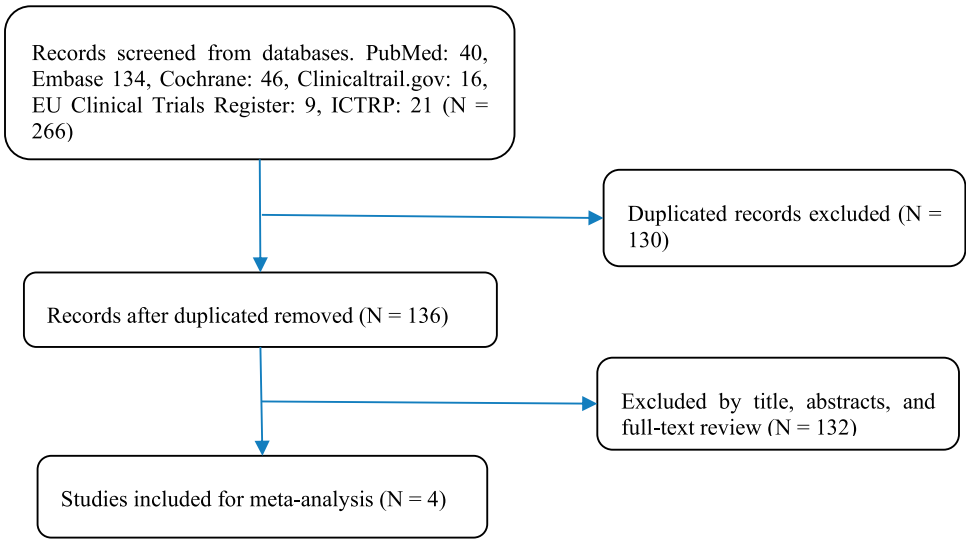

FIGURE 1
Flowchart of the study selection process.

Chi-squared-based Cochran's Q statistic and I^2. The heterogeneity was considered significant when the $p < 0.10$ or $I^2 > 50\%$. The fixed-effect model was used when data were homogenous, and the random-effect model was used when data were significantly heterogeneous. A sensitivity analysis was conducted using a leave-one-out approach.

3 Results

3.1 Search and study characteristics

A flow diagram of the study selection is presented in Figure 1. The search program yielded 266 references from PubMed (N = 40), Embase (N = 134), Cochrane Library (N = 46), Clinical Trials (N = 16), EU Clinical Trials Register (N = 9), ICTRP (N = 21). After excluding 130 duplicates, the remaining 136 articles were screened. Four multicenter, intention-to-treat RCTs published between 2019 and 2021 met the inclusion criteria and were included in the systematic review and meta-analysis. Three of the four studies were double-blind, and one was open-label (Table 1). All four studies were conducted in multiple countries. Among the 347 participants enrolled, 193 patients received Trilaciclib plus chemotherapy (experimental group), 154 patients received chemotherapy alone (control group), 169 patients were male, and 178 patients were female (Table 2). Weiss's study consists of part 1 (open-label, dose-finding) and part 2 (RCT, double-blind, placebo-controlled); only part 2 patients were included. In Weiss's study, two patients were excluded from data analysis for violation of study procedures, so the number of patients included for analysis in the experimental and control groups was

TABLE 2 characteristics of enrolled patients.

Study, year published	Group	Patient number	Baseline						
			Sex		Region		Age/years		
			Female	Male	USA	EX-USA	Median	18 to <65	≥65
J. M. Weiss, 2019	Control	37	11	27	39	38	66	17	21
	Experimental	38	12	27			64	20	19
Davey Daniel, 2020	Control	53	19	34	20	34	64 (46–83)	27	27
	Experimental	54	13	41	22	31	65 (45–81)	27	26
Lowell L. Hart, 2021	Control	29	12	17	18	11	64 (47–82)	18	11
	Experimental	32	10	22	14	18	62 (47–77)	20	12
Antoinette R Tan, 2019	Control	34	34	0	28	6	55 (43–64)	26	8
	Experimental	33	32	1	28	5	55 (47–66)	24	9
	Experimental	35	35	0	27	8	55 (49–65)	26	9

192 and 153, respectively. In Tan's study, two subgroups with different schedules of Trilaciclib administration (on days 1, 8, and 2, 9, respectively) were designed and analyzed independently. Trilaciclib was administered to patients at the recommended dose of 240 mg/m^2 0–3 days before chemotherapy started. Dose modifications were allowed for chemotherapy but not for Trilaciclib. All patients were diagnosed with SCLC or breast cancer. In the control group, the four studies used gemcitabine/carboplatin (G/P) therapy, etoposide/carboplatin/atezolizumab (E/P/A) therapy, etoposide/carboplatin (E/P) therapy and topotecan, respectively. Three studies focused on SCLC, with the other targeting metastatic triple-negative breast cancer. Prophylactic administration of ESAs or G-CSF was prohibited in cycle 1 to avoid interference with the results, but therapeutic ESAs or G-CSF usage was allowed in all cycles. The risk of bias in the included studies is presented in Figure 2, 3. Tan's study was found to have a high risk of bias in the domains of blinding of participants and performance and blinding of outcome assessment. All trials were designed and conducted in accordance with the Declaration of Helsinki and International Council for Harmonization Good Clinical Practice guidelines.

3.2 Clinical response

According to the results, 37 patients (19.3%) in the experimental group and 79 patients (42.2%) in the control group experienced SN (Figure 4A, OR = 0.31, 95% CI = 0.19–0.50, I^2 = 81%), and 4 (3.22%) and 8 (6.72%) patients in the experimental and control group experienced FN respectively (Figure 4B, OR = 0.47, 95% CI = 0.15–1.54, I^2 = 0%). Moreover, the DSN in the experimental group is significantly shorter than in the control group (Figure 4C, Mean Difference −1.36 days, 95% CI = −2.07–0.64, I^2 = 92%), implying that administration of Trilaciclib prior to chemotherapy efficiently reduced the CIM-related SN and FN and shortened the DSN during treatment.

ESA was administered to 5 patients (4.03%) in the experimental group versus 14 patients (11.8%) in the control group (Figure 5A, OR = 0.31, 95% CI = 0.11–0.90, I^2 = 0%). The percentage of patients receiving G-CSF in the experimental and control groups was 37.0% and 53.5%, respectively (Figure 5B, OR = 0.52, 95% CI = 0.34–0.78, I^2 = 79%). The proportion of patients receiving therapeutic use of ESAs and G-CSF was statistically lower in the experimental group than in the control group.

The percentage of patients with grade 3/4 anemia (Figure 6A, 20.5% vs. 38.2%, OR = 0.38, 95% CI = 0.24–0.62, I^2 = 0) and leukopenia (Figure 6B, OR = 0.31, 95% CI = 0.14–0.71, I^2 = 13%) was significantly lower in the experimental group than in the control group, in accordance with the proportion of patients receiving RBC transfusions (Figure 6C, 19.8% vs. 29.9%, OR = 0.56, 95% CI = 0.35–0.91, I^2 = 0) on/after week 5. There are also fewer patients experiencing grade 3/4 thrombocytopenia (Figure 7A, OR = 0.45, 95% CI = 0.27–0.75, I^2 = 47%) in the experimental group than in the control group. While the proportion of patients with platelet transfusions was identical in both groups (Figure 7B, 10.4% vs. 10.2%, OR = 1.00). It could thus be concluded that Trilaciclib reduced the occurrence of severe anemia, leukopenia, and thrombocytopenia and the need for RBC transfusions but had no impact on platelet transfusions.

The influence of Trilaciclib on the ORR, OS, and PFS is shown in Figure 8. As can be seen, the ORR (OR = 1.12, 95% CI = 0.71–1.77, I^2 = 0%), OS (Mean Difference −0.11, 95% CI = −0.58 – 0.36, I^2 = 73%), and PFS (Mean Difference 0.88, 95% CI = 0.73–1.04, I^2 = 96%) of the two groups were identical. Moreover, fewer patients experienced chemotherapy dose delays/reductions in the Trilaciclib arm than in the placebo arm, which helps to ensure the delivery of complete cycles of chemotherapy regimens. Administration of Trilaciclib showed no negative impact on the antitumor activity of chemotherapy treatments.

A statistical analysis of other drug-related AEs like vomiting, nausea, diarrhea, and fatigue is presented in Figure 9. No clinically relevant increase in toxicity was reported. The incidence of these AEs in both groups was identical, and grade 3/4 of these events were rare. No Trilaciclib-related grade 3/4 SAEs occurred, demonstrating that Trilaciclib has an acceptable safety profile.

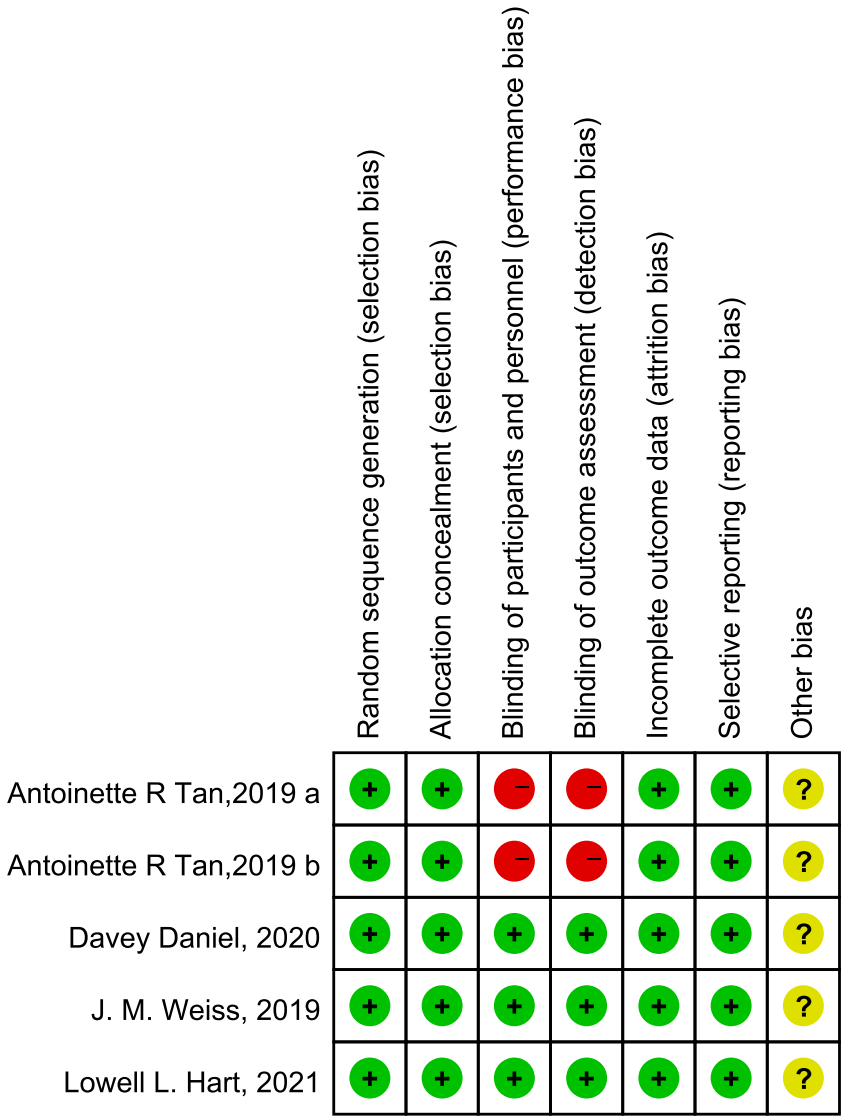

FIGURE 2
Risk of bias summary.

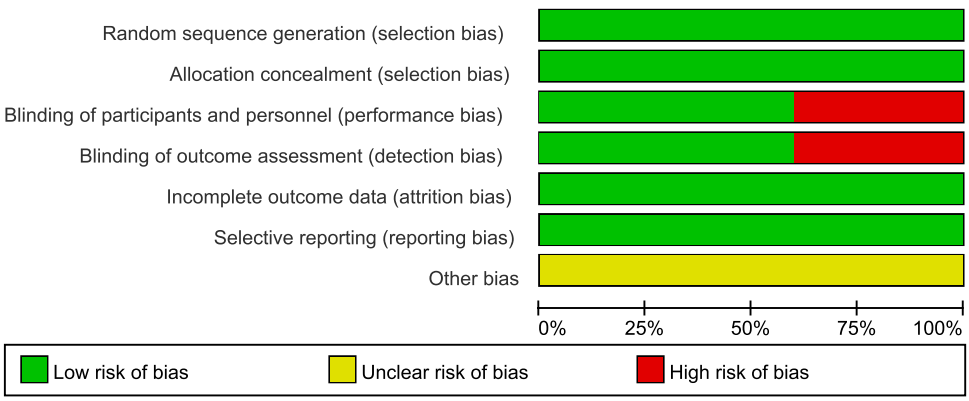

FIGURE 3
Risk of bias graph.

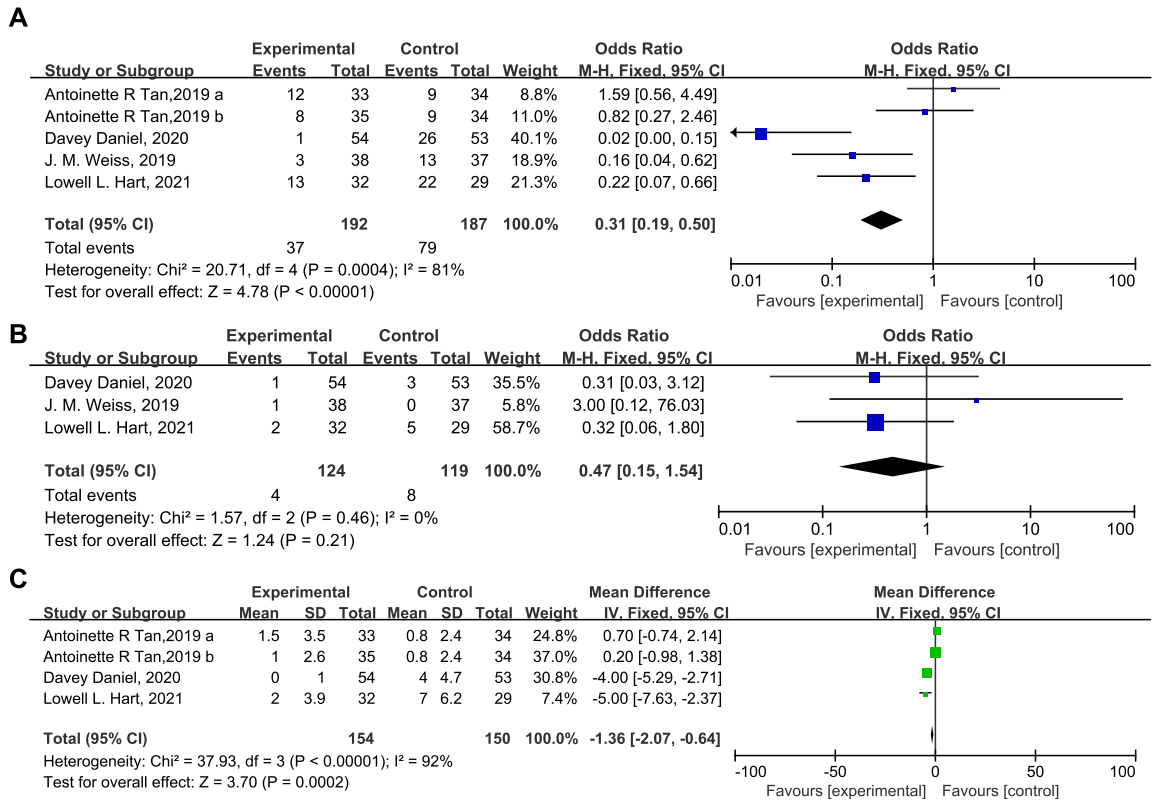

FIGURE 4
The statistical difference of SN **(A)**, FN **(B)**, and DSN **(C)** in the experimental and control group.

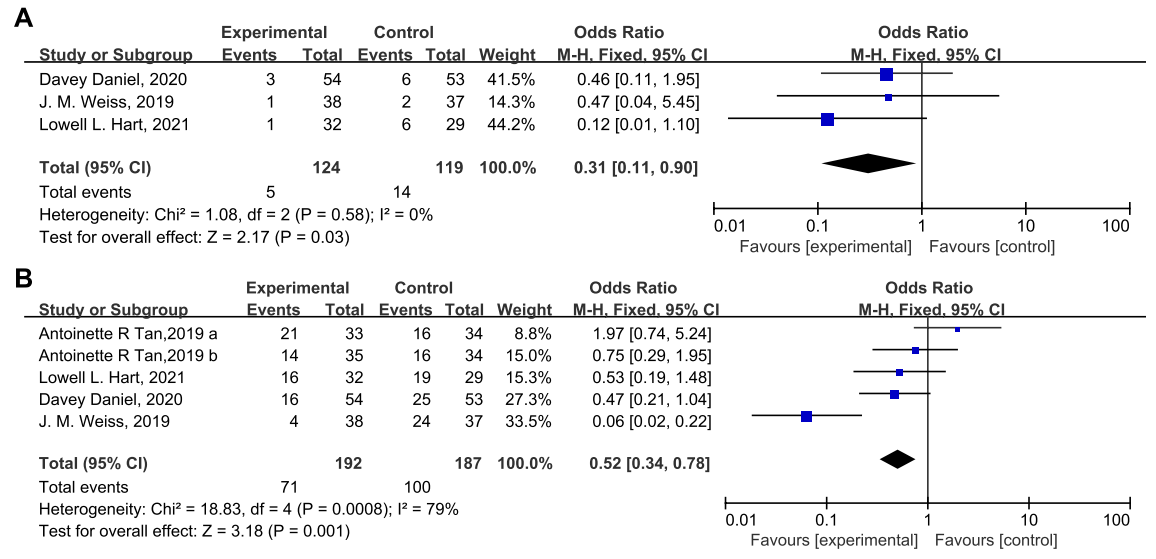

FIGURE 5
Therapeutic use of ESA **(A)** or G-CSF **(B)** in the experimental and control group.

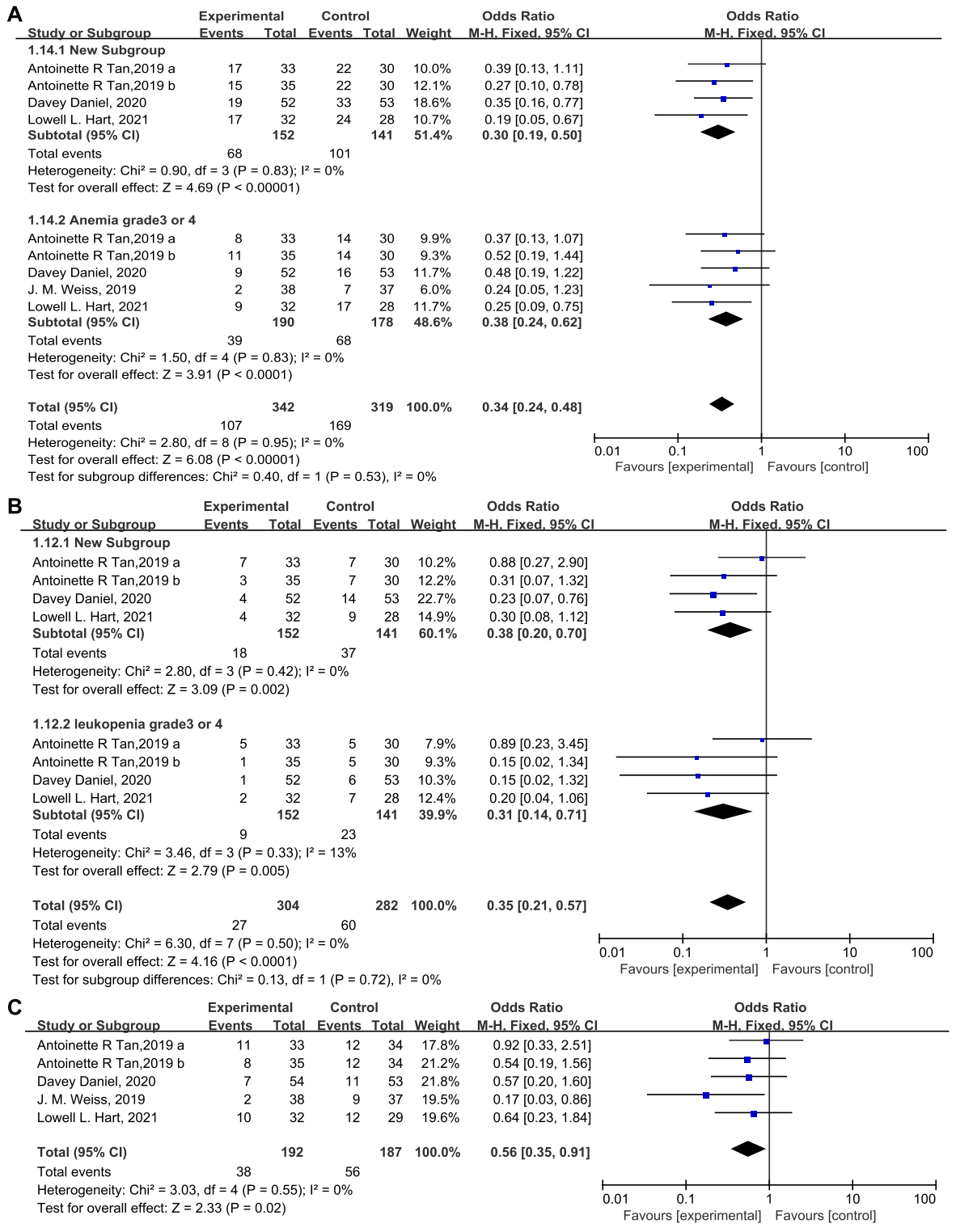

FIGURE 6
The occurrence of anemia **(A)** and leukopenia **(B)** and the proportion of patients with RBC **(C)** transfusions in the experimental and control group.

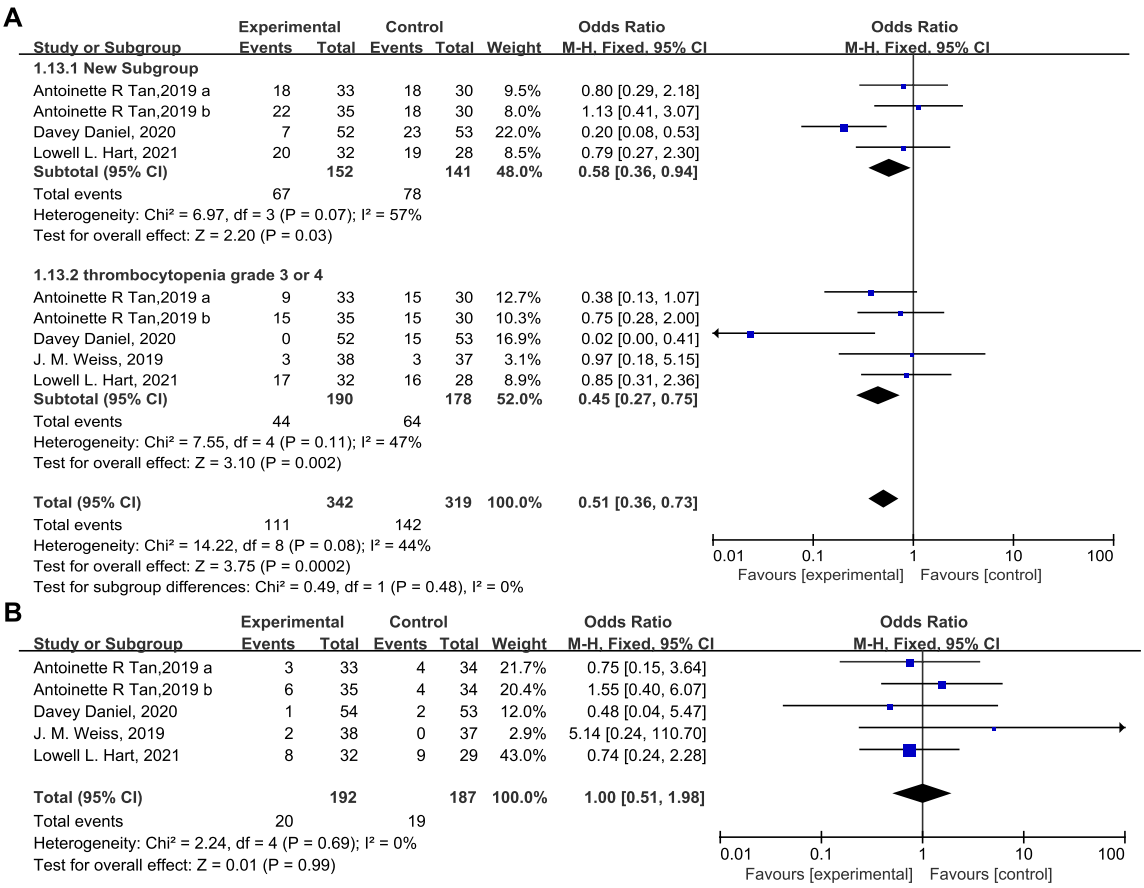

FIGURE 7
The occurrence of thrombocytopenia **(A)** and the proportion of patients with platelet transfusions **(B)** in the experimental and control group.

4 Discussion

Neutropenia and anemia are the most common side effects of CIM that are detrimental to chemotherapy treatments and are increasingly recognized as an important clinical issue that needs to be more efficiently managed. Trilaciclib was the first drug approved by the FDA to prevent CIM. By transiently arresting CDK4/6-dependent cells (like HSPCs and lymphocytes) in the G1 phase of the cell cycle, Trilaciclib protected these cells from cytotoxic chemotherapy and favorably altered the tumor immune microenvironment (Lai et al., 2020). Moreover, Trilaciclib has been shown to increase tumor cells' sensitivity to immune checkpoint inhibitors and prolong the duration of the antitumor responses in preclinical models (Deng et al., 2018; Lai et al., 2020). This supports the clinical trial of combining Trilaciclib with chemotherapy in patients with cancer. As SCLC tumor cells replicate independently of the CDK4/6 pathway, it is reasonable to conclude that Trilaciclib would achieve its efficacy without undermining the cytotoxic effect of chemotherapy agents on tumor cells, as has been demonstrated in multiple preclinical and clinical trials (Roberts et al., 2020).

In this meta-analysis of data from four phase 2 RCTs in patients with ES-SCLC and metastatic triple-negative breast cancer, administration of Trilaciclib prior to chemotherapy significantly reduced the occurrence of SN and FN and shortened the DSN during treatment. The use of supportive-care interventions like the administration of ESAs, G-CSF, and RBC transfusions on/after week 5 was also statistically reduced. Given the restricted use of ESAs and limited blood supplies in the context of COVID-19, this is especially helpful in relieving patients and the healthcare system from CIM-related anemia (Bohlius et al., 2019). Meanwhile, both groups' OS, PFS, and ORR were identical, implying that Trilaciclib protected patients from CIM without compromising the clinical benefits of chemotherapy treatments or bringing other unexpected side effects. The median age of the included patients was >55 years old. Considering that elderly patients were more frequently associated with CIM, the clinical benefit of Trilaciclib was more convincing. Trilaciclib showed its potential as a new standard of supportive care for patients receiving myelosuppressive chemotherapy treatments.

Though with encouraging outcomes, there are some limitations in this study. The first is the relatively small patient population, which may reduce the ability to detect minor potential statistically significant differences in clinical outcomes, AEs, and SAEs. Moreover, Trilaciclib showed its clinical efficacy in reducing the occurrence of CIM in treating SCLC in three clinical trials. Still, the metastatic triple-negative breast cancer trial observed no improvement in myelosuppression endpoints. Whether this is about gender differences, the type of cancer, or chemotherapy regimens needs to be determined. This underscores the need to

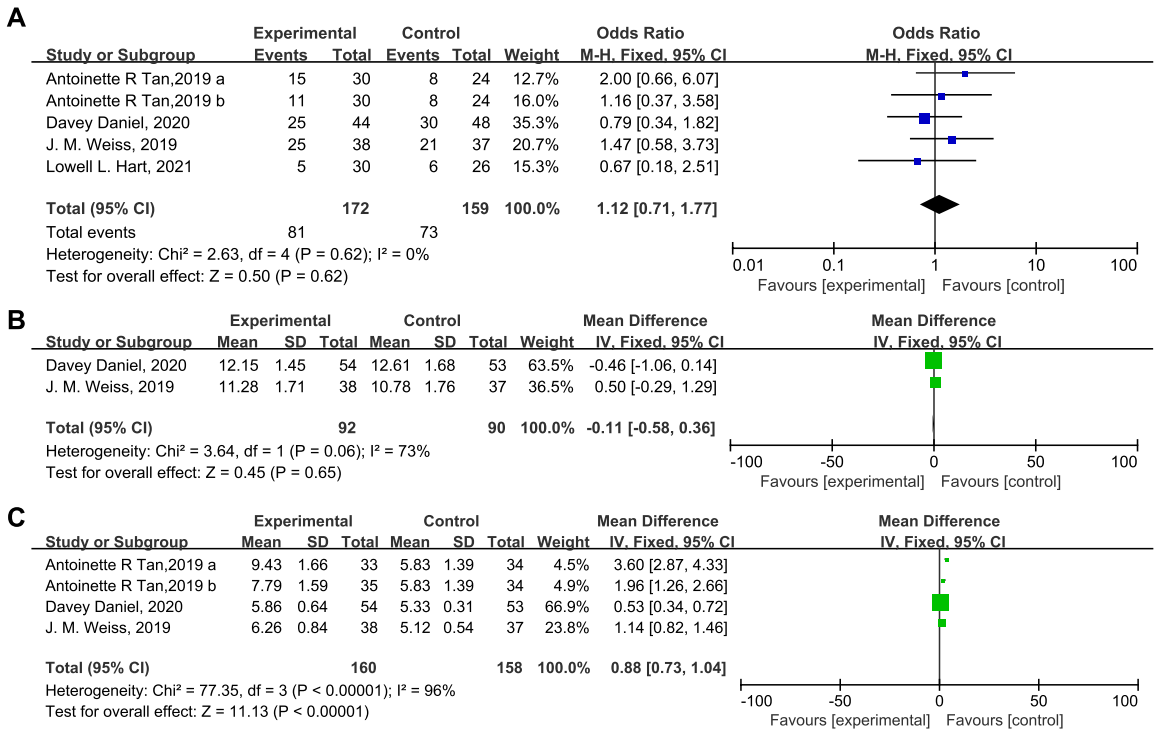

FIGURE 8
The impact of Trilaciclib on the ORR **(A)**, OS **(B)**, and PFS **(C)** in the experimental and control group.

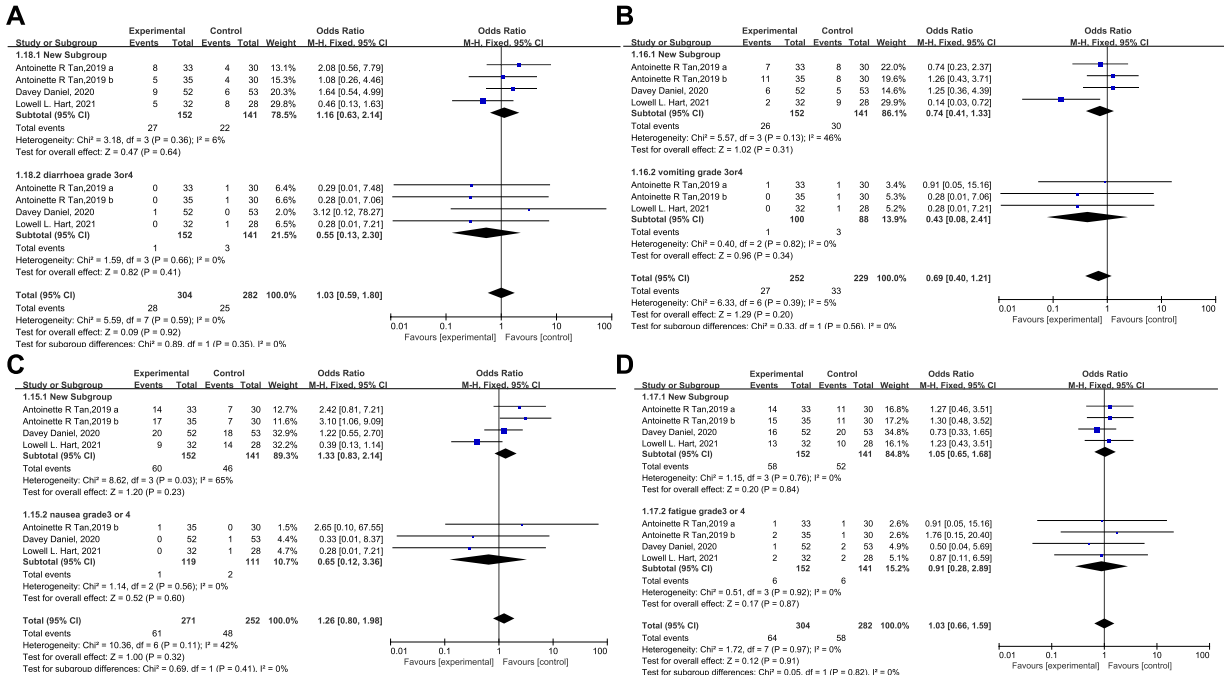

FIGURE 9
The occurrence of diarrhea **(A)**, vomiting **(B)**, nausea **(C)**, and fatigue **(D)** in the experimental and control group.

explore this difference's potential causes to confirm the clinical benefits of Trilaciclib further.

Together with these results, Trilaciclib demonstrated its efficacy in relieving patients from CIM-related side effects and improving the overall safety profile of myelosuppressive chemotherapy without inducing other unexpected side effects. These findings also support further clinical trials in a larger population and with more chemotherapy regimens in multiple types of cancers to demonstrate its clinical benefits.

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.

Author contributions

JQ and DS were involved in the literature search and selected the studies. JQ and PJ extracted and analyzed the data. FL drafted the manuscript. NS performed the study design and revised the manuscript. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. 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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Identification and prediction of immune checkpoint inhibitors-related pneumonitis by machine learning

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Background: Immune checkpoint inhibitor (ICI)-related pneumonitis (IRP) is a common and potentially fatal clinical adverse event. The identification and prediction of the risk of ICI-related IRP is a major clinical issue. The objective of this study was to apply a machine learning method to explore risk factors and establish a prediction model.

Methods: We retrospectively analyzed 48 patients with IRP (IRP group) and 142 patients without IRP (control group) who were treated with ICIs. An Elastic Net model was constructed using a repeated k-fold cross-validation framework (repeat = 10; k = 3). The prediction models were validated internally and the final prediction model was built on the entire training set using hyperparameters with the best interval validation performance. The generalizability of the final prediction model was assessed by applying it to an independent test set. The overall performance, discrimination, and calibration of the prediction model were evaluated.

Results: Eleven predictors were included in the final predictive model: sindillizumab, number of ≥2 underlying diseases, history of lung diseases, tirelizumab, non-small cell lung cancer (NSCLC), percentage of $CD4^+$ lymphocytes, body temperature, KPS score ≤70, hemoglobin, cancer stage IV, and history of antitumor therapy. The external validation of the risk prediction model on an independent test set of 37 patients and showed good discrimination and acceptable calibration ability: with AUC of 0.81 (95% CI 0.58–0.90), AP of 0.76, scaled Brier score of 0.31, and Spiegelhalter-z of −0.29 (P-value:0.77). We also designed an online IRP risk calculator for use in clinical practice.

Conclusion: The prediction model of ICI-related IRP provides a tool for accurately predicting the occurrence of IRP in patients with cancer who received ICIs.

KEYWORDS

immune checkpoint inhibitors, pneumonitis, risk prediction, machine learning, risk factors

1 Introduction

Immune checkpoint inhibitors (ICIs) are a new class of anticancer drugs that activate T-cell-mediated immune responses against tumor cells (1). Therapeutically blocking inhibitory molecules include cytotoxic T-lymphocyte antigen 4 (CTLA4) inhibitors, programmed cell death 1 (PD1) inhibitors, and programmed cell death 1 ligand (PD-L1) inhibitors (2). Trials have confirmed the clinical efficacy of ICIs in various advanced malignancies (2), and ICIs are emerging as a first-line treatment for some advanced cancers (2, 3). ICIs can result in a special set of adverse events termed immune-related adverse events (irAEs) (4, 5). IrAEs occur in all tissues and organs, most commonly in the lungs, skin, and liver. Common fatal irAEs are pneumonitis, myocarditis, colitis, hepatitis, and neurological effects (6, 7). Immune-related pneumonitis (IRP) is a clinically common, serious, and potentially lethal irAE, which develops in approximately 3.5%–19% of ICI therapy cases and accounts for 35% of ICI-related deaths (7). IRP can result in a high rate of treatment discontinuation (8) and cause a major economic burden on cancer patients (9). IRP is difficult to diagnose and there is no gold standard for clinical diagnosis (7).

ICI-related IRP requires significant attention given its clinical severity and diagnostic challenges (10). Previous studies have demonstrated that identification and prediction of the risk of ICI-related IRP are major issues (7, 11). Early prediction of the risk of IRP would reduce safety risks and improve clinical benefits. Establishing a prediction model is an effective way to achieve early prediction of the IRP. Machine learning is a new artificial intelligence method, which has been widely used to explore predictive factors and establish prediction models (12, 13). However, to date, no study has attempted to develop predictive models for IRP. In this study, we aimed to establish a prediction model to quantify individuals' IRP risk and provide an IRP risk prediction online calculator for clinical practice.

2 Materials and methods

2.1 Study data

We extracted electronic medical records from the Scientific Research Data Platform of patients discharged from Chongqing University Cancer Hospital between 1 January 2010, and 31 December 2021. Two clinicians were assigned to review the extracted electronic medical records independently and determine each patient's IRP status (IRP or non-IRP) and eligibility for this study. The diagnosis of IRP or non-IRP was based on the patient's clinical symptoms, laboratory test results, and the physician's clinical experience. Patients with a confirmed IRP diagnosis were classified into the IRP group. Patients who were diagnosed with pneumonitis but were not associated with an immune reaction or did not develop pneumonitis were classified into the non-IRP group. We first identified and included IRP cases, after which we randomly sampled non-IRP cases using a sample size four times the number of IRP cases. Patients were included in the IRP and non-IRP groups at a ratio of 1:4. The included patients were:1) aged 18 or above; 2) male and female; 3) diagnosed with cancer according to the pathological and clinical diagnosis; 4) treated with ICIs (only mono immunotherapy) in-hospital; and 5) never developed IRP before ICIs treatment. We excluded patients 1) whose treatment option was not ICIs and 2) patients receiving combination ICIs therapies.

This was a retrospective study and informed consent was not required. This study was approved by the Ethics Committee of the Chongqing University Cancer Hospital (CZLS2021042-A).

2.2 Study outcome and variables

The outcome of interest was IRP, defined as the manifestation of pneumonitis after ICI therapies related to immune reactions (14). Candidate predictors included the patients' demographic information (sex, age, height, weight, etc., which were measured before the assignment of ICI treatment), body temperature (refers to the forehead temperature measured by an Infrared Thermometer or armpit temperature measured by a Mercury Thermometer, and we selected the most recent result prior to ICIs initiation), disease situation (cancer types and cancer stage, etc.), treatment information (ICI drugs type, ICI dosage, number of combined drugs, previous treatment, etc.), and laboratory test data (blood routine examination, inflammatory, arterial blood gas, etc.), which were collected from the most recent laboratory test performed after cancer treatment and before the onset of IRP.

A complete list of the variables is provided in detail in Supplemental Appendix 1 (Table S1). Variables with a missing rate less than 15% were included. The handling of missing data is described in detail in Supplemental Appendix 2 (Table S3) and the preprocessing section in Supplemental Appendix 3.

2.3 Statistical analysis

All potential predictors were summarized and stratified according to the IRP status. Continuous and categorical variables were described as median (IQR) and frequency (percentage), respectively. Univariate analyses of each predictor between the IRP and non-IRP groups were conducted, continuous variables were assessed using the Kruskal–Wallis test, and categorical variables were analyzed using the chi-squared test or Fisher's exact test, as appropriate. The median or mode was used to impute missing data. Stratified sampling was used to divide the working dataset into two parts at a ratio of 8:2 (called the training and test sets, respectively). Subsequently, a multivariable risk prediction model was developed on the training set using the Elastic Net under a repeated k-fold cross-validation (repeats = 10; k = 3) framework. Specifically, for each combination of hyperparameters, the training set was randomly partitioned into three roughly equal sized parts; one part was left as the validation set, and the model was built on the remaining parts. The leave-out modeling process was conducted recursively until each part was treated as a validation set. The cross-validation modeling process was repeated 10 times, and the performance was evaluated on 30 validation sets. This procedure was repeated using different hyperparameter settings (we tuned 100 combinations of hyperparameters; the values are provided in Supplemental Appendix 3). The final prediction model was built on the entire training set using hyperparameters that yielded the best internal validation performance. Furthermore, the final prediction model was applied to external data (i.e., the test set) for external validation. The detailed modeling process is provided in Supplemental Appendix 3 (Figure S2).

The overall performance of the model was evaluated using the scaled Brier scores (SBrSs). Model discrimination was assessed using the area under the ROC curve (AUC), whose 95% confidence interval was obtained using bootstrapping, and calibration was evaluated using average precision (AP, the area under the precision-recall curve) and Spiegelhalter-z statistics. SHAP (Shapley Additive exPlanations) values was utilized to visualize the variable importance. Calibration plots and risk stratification results were generated to examine model performance in different sub-risk groups. An online calculator was developed using R shiny, which allows clinicians and cancer survivors to calculate personalized IRP risks. All performance matrices were computed on the validation and test sets, and the metrics reported in the *Results* section were cross-validated (Figure 1).

Statistical analyses were conducted in R (version 4.1.2) and *P*-values less than 0.05 were considered statistically significant, and all tests were two-tailed.

The code generating all results is publicly available [https://github.com/gongli0707/IRP-prediction].

3 Results

3.1 Study patients

According to the inclusion and exclusion criteria, 190 cases were identified from the Scientific Research Data Platform of Chongqing University Cancer Hospital, which included 48 IRP cases and 142 non-IRP cases. The screening flow is illustrated in Figure 2.

The median ages of IRP and non-IRP patients were 61.00 [IQR: 54.75–67.00] years and 58.00 [IQR: 52.00, 67.00] years, respectively. The number of males was higher than that of females in both the groups. The baseline body temperature showed a statistical difference between the IRP and non-IRP groups (the distribution

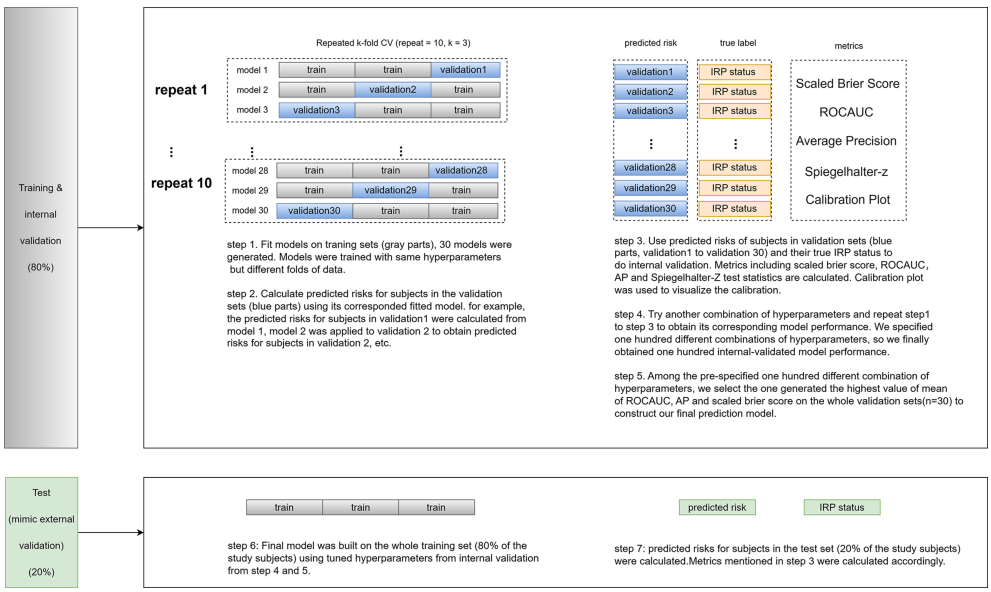

FIGURE 1
The workflow of the IRP prediction model building.

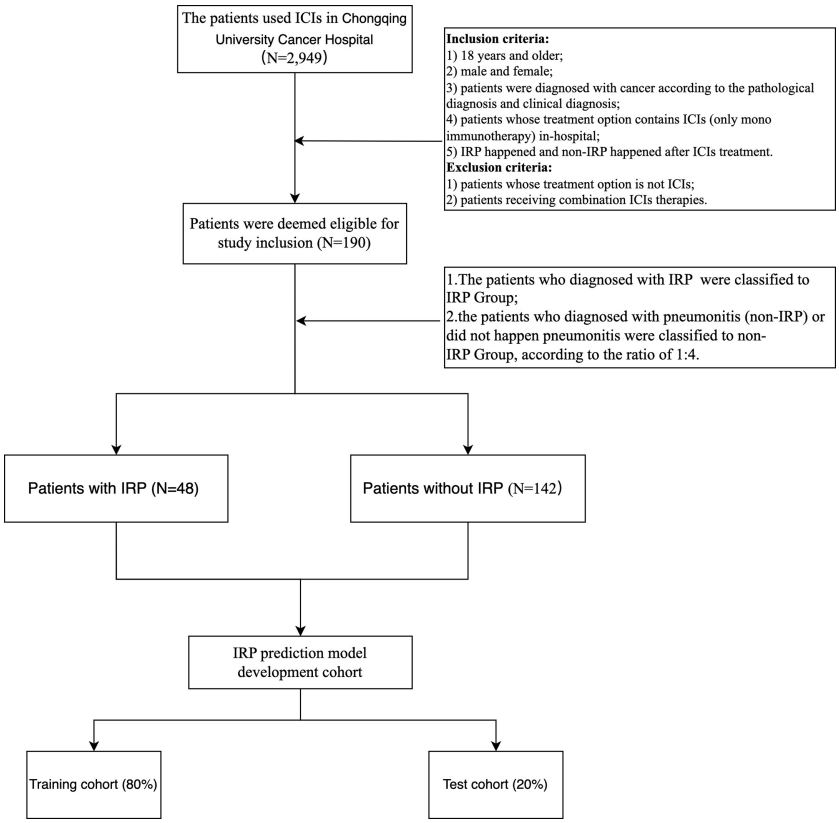

FIGURE 2
The flow chart of this study.

features are provided in Supplemental Appendix 1; Figure S1). The most common stage of cancer was stage IV, followed by stage III in all cohorts. Karnofsky performance status (KPS) score was median in 80. The relationship between these factors and the occurrence of IRP are further screened in the following sections. We did not find significant differences in underlying diseases (hypertension, diabetes, CHD, viral hepatitis, and lung-related diseases) between the IRP and non-IRP groups. However, the number of underlying diseases was statistically significant (P <0.05). IRP risk was statistically different in patients treated with PD-1 and PD-L1. Patients in the PD-1 group were less likely to develop IRP than those in the PD-L1 group. Moreover, the combination of non-antitumor drugs, history of radiation therapy, T lymphocyte count, and percentage of basophils might have contributed to IRP outcome (P <0.05) (Table 1).

3.2 IRP risk prediction

Eighteen variables (a list can be found in Table 1) were found associated with ICI-related IRP in the univariate analyses. Under the repeated cross-validation framework, we tuned the hyperparameters and finally determined that the model with an alpha of 1.000 and lambda of 0.026 generated the best model performance. The final prediction model was trained on the full training set by using these parameters. The final prediction model included 11 predictors: sindillizumab, ≥2 underlying diseases, history of lung diseases, tirelizumab, NSCLC, percentage of $CD4^+$ lymphocytes, body temperature, KPS score ≤70, hemoglobin, cancer stage IV, and history of antitumor therapy. The coefficients of the 11 predictors are presented in Supplemental Appendix 3 (Table S5). An online ICI-related IRP risk calculator was developed using our final predicted model and can be accessed through https://lin-yu.shinyapps.io/IRPcalculator/.

3.2.1 Model performance

The final prediction model had adequate discrimination, with a cross-validated AUC of 0.81 (95% CI: 0.79–0.84) over the validation sets. The AP value was considerably higher than the event rate (AP = 0.58; event rate = 25%), and the Spiegelhalter-z was 0.34 (P-value: 0.74), indicating good calibration. A predictive model was applied to the test set for external validation. The AUC estimate was 0.81 (95% CI: 0.55–0.90), the AP was 0.68, and the Spiegelhalter-z was −0.29 (P-value: 0.77) (Table 2). Therefore, we conclude that our predictive model has the potential for IRP risk prediction.

3.2.2 Variable importance

We used feature importance and SHAP plots to visualize the variable importance (Figure 3). Predictors are shown in order of global feature importance, with the first being the most important and the last being the least important. We also used the SHAP value to visualize the variable importance and direction of the association

TABLE 1 The results of characteristics of patients by univariate analysis.

	IRP (n = 48)	Non-IRP (N = 142)	*P*-value
Sex			0.331
Male	41 (85.4)	110 (77.5)	
Female	7 (14.6)	32 (22.5)	
Age (y)	61.00 [54.75, 67.00]	58.00 [52.00, 67.00]	0.358
BMI	22.98 [20.82, 25.38]	23.56 [21.48, 25.08]	0.592
Body Temperature (°C)	36.60 [36.50, 36.80]	36.50 [36.30, 36.70]	**0.045**
Systolic blood pressure	121.50 [108.25, 129.00]	124.00 [112.25, 133.75]	0.346
Diastolic blood pressure	77.00 [70.00, 83.75]	79.50 [71.00, 85.00]	0.442
Smoking (yes)	31 (64.6)	72 (50.7)	0.133
Drinking (yes)	13 (27.1)	27 (19.0)	0.327
KPS score			0.169
≤70	17 (35.4)	31 (21.8)	
80	88 (62.0)	24 (50.0)	
≥90	7 (14.6)	23 (16.2)	
Cancer stage			0.708
I	0 (0.0)	1 (0.7)	
II	1 (2.1)	3 (2.1)	
III	18 (37.5)	42 (29.8)	
IV	29 (60.4)	95 (67.4)	
Cancer category			0.013
NSCLC	42 (87.5)	96 (67.6)	
Non NSCLC[1]	6	46	
Type of underlying diseases			
Hypertension	11 (22.9)	20 (14.1)	0.228
Diabetes	10 (20.8)	16 (11.3)	0.154
Coronary Heart Disease	4 (8.3)	7 (4.9)	0.474
Viral Hepatitis	3 (6.2)	13 (9.2)	0.765
Lung-related disease	8 (16.7)	18 (12.7)	0.651
Number of underlying diseases			**<0.001**
0	6 (12.5)	62 (43.7)	
1	15 (31.2)	52 (36.6)	
≥2	27 (56.2)	28 (19.7)	
History of lung diseases	2 (4.2)	35 (32.7)	**0.004**
ICI drugs			**0.047**
PD-L1	5 (10.4)	4 (2.8)	
PD-1	43 (89.6)	138 (97.2)	
ICIs drugs			**<0.001**
Attilizumab	2 (4.2)	3 (2.1)	
Carrilizumab	19 (39.6)	36 (25.4)	

(Continued)

TABLE 1 Continued

	IRP (n = 48)	Non-IRP (N = 142)	*P*-value
Tirelizumab	3 (6.2)	31 (21.8)	
Nevirumab	2 (4.2)	6 (4.2)	
Perbolizumab	11 (22.9)	12 (8.5)	
Toripalimab	5 (10.4)	18 (12.7)	
Sindillizumab	0 (0.0)	33 (23.2)	
others	6 (12.5)	2 (1.4)	
ICIs drug dosage (mg)	200.00 [200.00, 200.00]	200.00 [200.00, 200.00]	0.158
First time for immunotherapy (yes)	45 (93.8)	129 (90.8)	0.765
Course of cancer treatment	4.00 [3.00, 7.00]	5.00 [3.00, 7.00]	0.222
Number of other antitumor drugs			0.153
0	8 (17.0)	35 (24.6)	
1	14 (29.8)	21 (14.8)	
2	24 (51.1)	83 (58.5)	
3	1 (2.1)	2 (1.4)	
≥4	0 (0.0)	1 (0.7)	
History of other antitumor drugs exposure (yes)	39 (83.0)	107 (75.4)	0.546
Number of non-antitumor drugs			**0.027**
0	13 (50.0)	102 (74.5)	
1	3 (11.5)	17 (12.4)	
2	4 (15.4)	7 (5.1)	
3	3 (11.5)	5 (3.6)	
4	2 (7.7)	3 (2.2)	
5	1 (3.8)	1 (0.7)	
≥6	0 (0.0)	2 (1.5)	
History of non-antitumor drugs exposure (yes)	13 (50)	35 (25.5)	**0.005**
Surgery (yes)	11 (26.2)	42 (29.6)	0.817
History of radiation therapy (yes)	27 (64.3)	48 (33.8)	**0.002**
History of chemotherapy (yes)	32 (66.7)	78 (54.9)	0.210
Number of previous anti-tumor drugs			0.070
0	17 (35.4)	53 (40.2)	
1	1 (2.1)	3 (2.3)	
2	28 (58.3)	51 (38.6)	
3	2 (4.2)	23 (17.4)	
4	0 (0.0)	1 (0.8)	
≥5	0 (0.0)	1 (0.8)	
History of anti-tumor drugs exposure (yes)	31 (64.6)	89 (62.7)	0.866
$CD4^+$ lymphocyte count	264.50 [186.75, 490.00]	403.50 [256.25, 582.75]	0.054

(Continued)

TABLE 1 Continued

	IRP (n = 48)	Non-IRP (N = 142)	*P*-value
Percentage of $CD4^+$ lymphocytes	31.05 [25.62, 41.05]	35.20 [28.33, 44.88]	0.102
$CD8^+$ lymphocyte count	260.50 [189.50, 356.75]	308.00 [183.75, 406.50]	0.249
Percentage of $CD8^+$ lymphocytes	28.20 [21.52, 37.38]	26.75 [20.52, 33.22]	0.565
T lymphocyte count	570.00 [427.50, 867.25]	752.00 [554.50, 1049.50]	**0.049**
Percentage of T lymphocytes	67.60 [58.80, 75.55]	71.10 [62.00, 77.35]	0.292
B lymphocyte count	77.50 [31.75, 129.75]	90.00 [53.50, 141.50]	0.331
Percentage of B lymphocytes	7.90 [4.65, 13.22]	8.50 [5.35, 12.85]	0.629
NK cell count	201.50 [124.00, 297.25]	206.00 [122.00, 289.50]	0.896
Percentage of NK cell	18.95 [14.78, 31.52]	18.60 [12.45, 27.85]	0.488
Red blood cell	3.92 [3.38, 4.34]	3.84 [3.40, 4.17]	0.592
Hemoglobin	118.50 [103.75, 127.00]	116.50 [106.75, 129.00]	0.781
Hemameba	5.72 [4.30, 7.91]	5.17 [4.15, 6.56]	0.139
Percentage of lymphocytes	17.75 [11.18, 24.52]	20.25 [13.30, 27.77]	0.151
Percentage of monpcytes	9.20 [6.00, 12.35]	10.05 [7.60, 12.50]	0.370
Percentage of neutrophilic granulocyte	68.70 [59.65, 79.20]	66.40 [58.43, 74.35]	0.278
Percentage of eosinophils	1.00 [0.30, 3.80]	1.90 [0.60, 3.15]	0.379
Percentage of basophils	0.30 [0.10, 0.60]	0.40 [0.30, 0.70]	**0.034**
Blood platelet	184.00 [144.25, 256.50]	181.00 [139.50, 233.75]	0.354

1: non-NSCLC contains malignant melanoma, small cell lung cancer, nasopharyngeal carcinoma, cervical cancer, Hodgkin's lymphoma, ovarian cancer, diffuse large B-cell lymphoma, esophageal carcinoma, gastric cancer, bladder cancer and others. The bold values means less than 0.05.

(Figure 4). A positive SHAP value indicates a positive association between predictors and ICI-related IRP; likewise, a negative SHAP value corresponds to a negative association between predictors and ICI-related IRP. The SHAP plot indicated that the number of underlying diseases ≥2, NSCLC, KPS score ≤70, history of antitumor therapy, other ICI drugs, and body temperature were positively associated with IRP, whereas the remaining predictors were negatively related to IRP.

3.2.3 Calibration plots

Calibration plots were used to visualize the calibration (Figure 5). In the validation set, we observed great calibration for patients with predicted risk less than 0.8, and overestimated IRP risk in patients with predicted risk between 0.8 and 1.0, respectively. In the test set, we found that the model underestimated the risk in high IRP risk group and overestimated the IRP risk in the low-risk group. Taken together, the predicted probability risks in the subgroups were close to the observed proportion, suggesting that our model was well-calibrated.

3.2.4 Risk stratification

Using 5%, 20%, 50%, and 80% as cut-offs, the predicted probabilities of IRP were stratified into four risk categories: <5%, 5% to <20%, 20% to <50%, 50% to 80%, and ≥80%, each corresponding to a different level of risk, including low-, medium-low, medium, median-high, and high-risk groups. Table 3 shows that our model performed well with regard to the risk stratification. In the validation set, among 31 participants with a predicted IRP risk greater than 80%, 61% (19 out of 31) developed IRP; 325 participants with a predicted IRP risk of less than 0.05, 5% (15/325) of them developed IRP. Risk stratification in the test set indicated good calibration. Two and five of the 17 and 13

TABLE 2 Summary of performance of model on training and test datasets.

Data	AUC (95% CI)	AP	SBrS	Spiegelhalter-z (*p*-value)
Validation[1]	0.81 (0.79–0.84)	0.58	0.27	0.34 (0.74)
Test[2]	0.81 (0.55–0.90)	0.68	0.30	−0.29 (0.77)

[1]Validation data refers to thirty leave-out parts in repeated CV framework. [2]Test data is the 20% of the whole study subjects which was used to mimic an external data source for external validation.

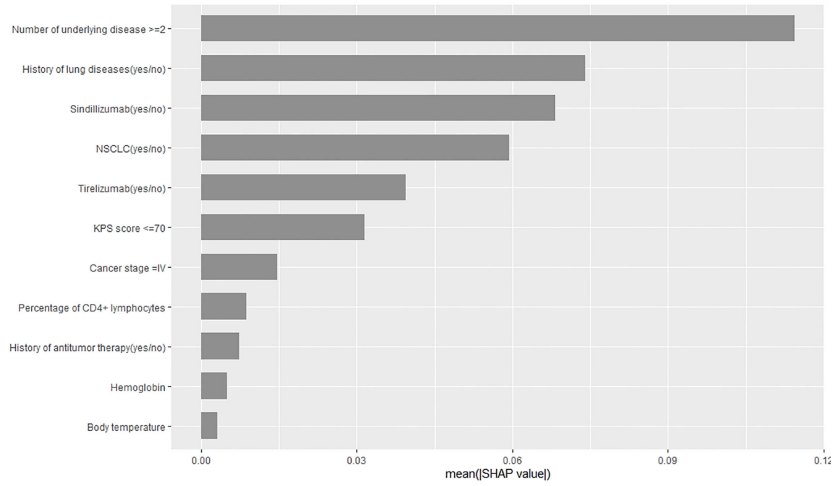

FIGURE 3
The rank of features in the prediction model by the degree of importance.

individuals who were predicted to be at medium-low and medium IRP risk, respectively, developed IRP.

4 Discussion

The utility of electronic medical records (EMR) has expanded from data storage to data utilization using various methods, which could guide clinical decisions and predict important outcomes (15). Establishing an ICI-related IRP prediction model using EMR and machine learning algorithms is an effective and low-cost approach. We identified potential IRP predictors such as the number of underlying diseases, ICI drugs (sindilizumab and tirelizumab), history of lung disease, NSCLC, percentage of $CD4^+$ lymphocytes, and body temperature, KPS score ≤70, hemoglobin, cancer stage IV, and history of antitumor therapy. We also developed and validated an IRP prediction model for patients with cancer, using the Elastic Net model. We further applied the final model to establish a user-friendly IRP risk calculation tool, in which personalized IRP risk could be calculated using relevant clinical information.

We found that the total number of underlying diseases was the most important risk factor for IRP. Patients with more than two underlying diseases might have a greater risk of developing IRP if they received ICI treatment. This phenomenon might contribute to the poor performance status (16). In our study, we found that hypertension, diabetes, coronary heart disease, viral hepatitis, and chronic obstructive pulmonary disease were the most common combination of diseases. Interestingly, we did not find that these combination diseases had statistically significant differences associated with IRP, which differs from previous studies. Some previous studies have suggested that pre-existing lung diseases, such as chronic obstructive pulmonary disease, might contribute to IRP,

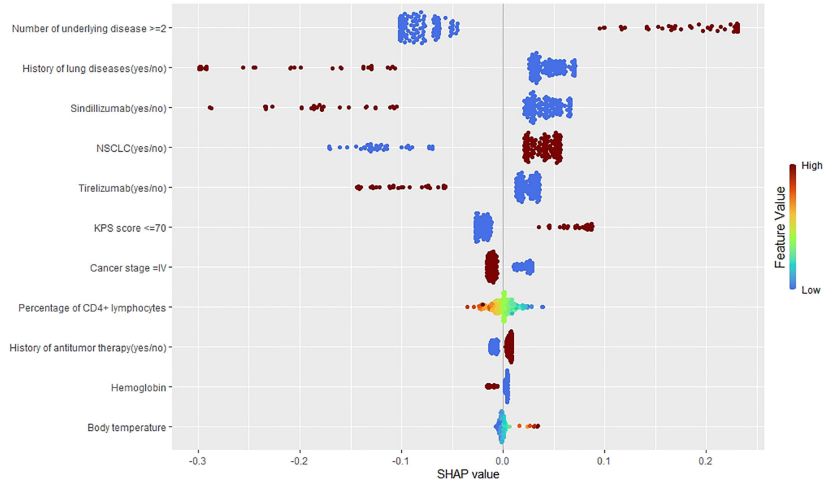

FIGURE 4
The SHAP value of features in the prediction model.

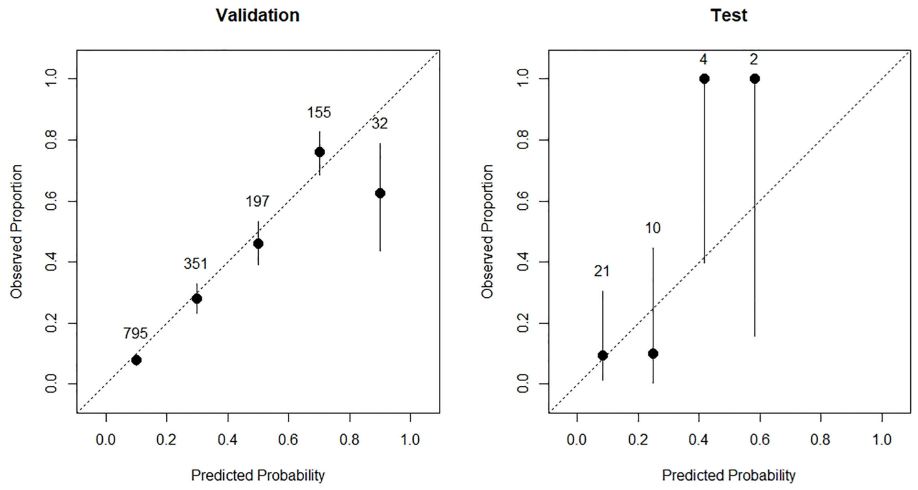

FIGURE 5
The calibration plots of validation cohort (left) and test cohort (right) of the prediction model.

but this was not confirmed by a statistically significant difference (7, 11, 17).

The incidence of IRP is affected by anti-PD-1 agents. In contrast to anti-PD-L1 and anti-CTLA-4 drugs, anti-PD-1 agents are more likely to cause adverse reactions in the lungs (7). However, we could not rule out the possibility that other ICIs could result in IRP in patients who underwent immunotherapy. In our study, we did not include data on combination immunotherapy. Previous studies have suggested that patients receiving combination immunotherapy are associated with a higher incidence of lung toxicity than patients receiving monotherapy (14, 18). Combination therapy with other antitumor drugs, such as chemotherapy, was the most common treatment, and there was no statistically significant difference between IRP and non-IRP. There is still limited evidence on the IRP of ICI combined with chemotherapy (19).

NSCLC was a risk factor for IRP, which was more common in lung cancer patients treated with ICIs than in patients with other cancers. Some studies have demonstrated that IRP has been repeatedly reported in NSCLC patients compared to patients diagnosed with melanoma and head and neck squamous cell carcinoma (17, 20). However, the biological mechanism of IRP in NSCLC is poorly understood. Dysregulated activation of T cells in peripheral lung tissue (21) and the predisposition of peritumoral lung tissue to irAEs (7) may play an important role.

For laboratory indexes, $CD4^+$ lymphocyte count after ICI treatment was negatively correlated with IRP; that is, a smaller $CD4^+$ lymphocyte count is related to higher IRP risk. $CD4^+$ lymphocytes are a subpopulation of T lymphocytes, and $CD4^+$ cell can cooperate with cytotoxic T lymphocytes contributing to the efficacy of immunotherapy (22). ICIs may increase the greater magnitude of T-cell proliferation or decrease $CD4^+$ cell-mediated immunosuppression (23). A lower count of $CD4^+$ cells may indicate an active immune response. IRP is an active inflammatory infiltrative lung disease associated with an immune response (8), and the count of $CD4^+$ lymphocyte cells can predict the risk of IRP.

In this study, we established a prediction model for the IRP of cancer patients using ICIs. Accordingly, an online calculation tool was developed. Users can upload relevant information to obtain the IRP risk immediately. An early understanding of the risks of IRP will improve the clinical benefit for patients. However, this study had some limitations. First, this was a retrospective study with a lack of prospective verification. This may introduce selection bias,

TABLE 3 The risk stratification of ICIs-related IRP in our prediction model.

	Validation		Test	
Risk groups	# of IRP event/ # of patients	PPV	# of IRP event/ # of patients	PPV
0.05 (low-risk)	15/325	0.05	0/5	0.00
5%–20% (medium-low)	48/466	0.10	2/17	0.12
20%–50% (medium)	146/470	0.31	5/13	0.38
50%–80% (medium-high)	162/238	0.68	2/2	1.00
>80% (high-risk)	19/31	0.61	0/0	/

PPV, positive predictive value; samples are 10 times that of the original training set, due to the repeated CV framework (repeat = 10). "#" means number and "/" means division sign.

specifically sampling bias, which may limit the generalizability of the results. In addition, the data selected from a single resource may not be representative of the characteristics of the general population. Therefore, the external validation in this study did not guarantee a good performance when applying the model to the general population.

Second, the small sample size may have caused imbalanced distributions of variables and biased the estimate of IRP risk. In our study dataset, we observed skewed distributions for sex and PD-1 therapy, where female patients and patients who underwent PD-L1 therapy were underrepresented. Our analysis revealed that there was no statistically significant difference in the sex distributions between the IRP and non-IRP groups and that sex was not a predictor of IRP, which is consistent with earlier meta-analyses and the findings of a real-world study (24). Although the proportion of patients who received PD-1 therapy in two comparison groups was statistically significant, PD-1 therapy was not a predictor of IRP risk. Interpretation of the observed statistical significance should be done with care, as the PD-1 therapy distribution in our study sample could be imbalanced and not representative of the true pattern of the data. To address this concern, we used a repeated CV framework to make full use of the data; however, the observed model performance in the test set should be interpreted with caution because of the limited sample size. Third, variables (such as Tumor Mutational Burden (25)) that were found to be associated with IRP could not be included in the prediction model because of the high missing rates. In the future, a higher-quality data, multicenter, larger sample, and prospective study is needed to optimize and prove the validity of the IRP prediction model before it can be used in a clinical setting.

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.

Author contributions

LG, JG, XS, LY, XC, and Y-SL contributed to conception and design of the study. LG and JG screened the data and organized the database. JG, XS, and LY performed the statistical analysis. LG, JG, XS, LY, and BL wrote the first draft of the manuscript. Y-SL and XC reviewed and modified the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Author LY and XS were employed by the company NanPeng Artificial Intelligence Research Institute Ltd.

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/fimmu.2023.1138489/full#supplementary-material

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Case Report: Replacement of PD-1 inhibitors with PD-L1 inhibitors in the treatment of squamous non-small-cell lung carcinoma

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Background: Immune checkpoint inhibitor (ICI)-associated cardiotoxicity is a relatively uncommon immune-related adverse effects (irAEs) with a high mortality rate. There are few recommendations for the replacement of different immune checkpoint inhibitors in domestic and international reports.

Case presentation: We report a case of a patient with squamous non-small cell lung carcinoma (squamous NSCLC) who developed cardiotoxicity after being treated with a programmed death-1 (PD-1) inhibitor and then changed to a PD-L1 inhibitor to continue the treatment. A significant benefit was observed after four cycles of immunotherapy, and no further cardiotoxicity occurred after the treatment was started.

Conclusion: This case demonstrates that myocardial damage induced by tislelizumab (PD-1 inhibitor) can be improved after switching to sugemalimab (PD-L1 inhibitor) and that antitumor immunotherapy is effective. This result may have important implications for optimizing immunotherapy management regimens in cancer patients.

KEYWORDS

tislelizumab, cardiotoxicity, sugemalimab, PD-L1 inhibitor, squamous NSCLC, case report

1 Introduction

Tislelizumab is a PD-1 inhibitor that enhances the human tumor immune response by specifically blocking the interaction between PD-1 and PD-L1. Based on clinical research evidence from RATIONALE 307, tislelizumab was approved by the National Medical Products Administration (NMPA) for the first-line treatment of advanced squamous NSCLC on 12/01/2021 (1). However, there were no clinical data and treatment included in the trial related to tislelizumab combination chemotherapy-related cardiotoxicity. This article reported a case of a squamous NSCLC patient who developed cardiotoxicity after

receiving one cycle of combination therapy using tislelizumab (PD-1 inhibitor), combined with nab-paclitaxel and carboplatin. Moreover, the combination therapy continued after the patient got better while tislelizumab was replaced with sugemalimab (PD-L1 inhibitors).

2 Case description

In March 2022, a 59-year-old man was admitted to the clinic with a persistent cough producing blood-streaked sputum for 6 months, which worsened over the last 2 months. He arrived with stable vital signs, without chest heartburn or pain, and without dizziness or palpitations (ECOG score: 1). The patient had a history of coronary heart disease for 10 years with one stent implanted in the circumflex, and cardiac ultrasound indicated that cardiac function was lower than normal. There was no previous history of hypertension or diabetes mellitus. CT scan with chest contrast revealed a right hilar mass enveloping the right pulmonary artery, and the right hilar lymph node was about 2.3 cm. A pathological tissue biopsy was performed by tracheoscopy to consider squamous carcinoma, which was finally diagnosed as stage IIIB squamous carcinoma of the right lung (pT4N2M0), and no surgical indication was available at this stage. A craniocerebral MRI enhancement scan and abdominal CT non-contrast scan did not reveal metastases. Cardiac ultrasound showed that the left ventricular ejection fraction (LVEF) was reduced with 50%. In addition, the electrocardiogram (ECG), cardiac enzymes, and liver and kidney functions were normal.

Subsequently, the patient was treated with nab-paclitaxel 300 mg + carboplatin 400 mg + tislelizumab (PD-1 inhibitor) 200 mg for one cycle. The treatment went well, and the patient had no discomfort. Two weeks later, the patient's cardiac enzymes were significantly elevated on clinic recheck: creatine kinase (CK) 195 U/L (reference: 0–173), CK isoenzyme 165 U/L (reference: 0–24), lactic dehydrogenase (LDH) 238 U/L (reference: 15–220). We considered the development of immunotherapy-related cardiotoxicity (G2); thus, the patient was administered trimetazidine to support myocardial nutrition. On the next day, the cardiac enzymes showed that CK isoenzyme decreased to 100 U/L, CK 212 U/L, and LDH 245 U/L. Cardiac ultrasound showed LVEF 43%, 7% lower than the baseline, so myocardial nutrition therapy was continued. Before the second cycle of treatment, all the cardiac enzymes returned to normal on recheck (Figure 1).

After extensive consideration of the necessity of treatment and the differences in the incidence of cardiotoxicity among different types of ICIs (2, 3), we decided to initiate one cycle of chemotherapy combined with PD-L1 inhibitor treatment after the cardiac enzymes had returned to normal. Specific dosage included nab-paclitaxel 300 mg + carboplatin 400 mg + sugemalimab (PD-L1 inhibitor) 1,200 mg. The patient was rechecked after a month; the results of troponin, CK, and LDH were normal, while that of CK isoenzyme was mildly increased (72 U/L). Four cycles of sugemalimab combined with chemotherapy later, cardiac enzymes and ECG remained normal, and LVEF increased from 43% to 55%. Treatment is currently ongoing. Tumor markers including carcinoembryonic antigen (CEA, from 5.45 ng/mL to 3.78 ng/mL), cytokeratin 19 fragment (CYFRA21-1, from 7.3 ng/mL to 2.21 ng/mL), and squamous cell carcinoma-associated antigen (SCC, from 1.05 ng/mL to 0.65 ng/mL) gradually decreased during the treatment. Review of CT scan with contrast for evaluation of the target lesion prompted the efficacy of the final treatment as PR (Figure 2). Subsequently, the patient continued to be followed up and the lesion remained stable without recurrence of cardiotoxicity.

3 Discussion

In this case, the patient had a history of coronary artery disease, which increased the risk of immunotherapy-related cardiotoxicity (4). The patient developed cardiotoxicity after one cycle of tislelizumab combined with chemotherapy, and the subsequent treatment options were adjusted from tislelizumab to

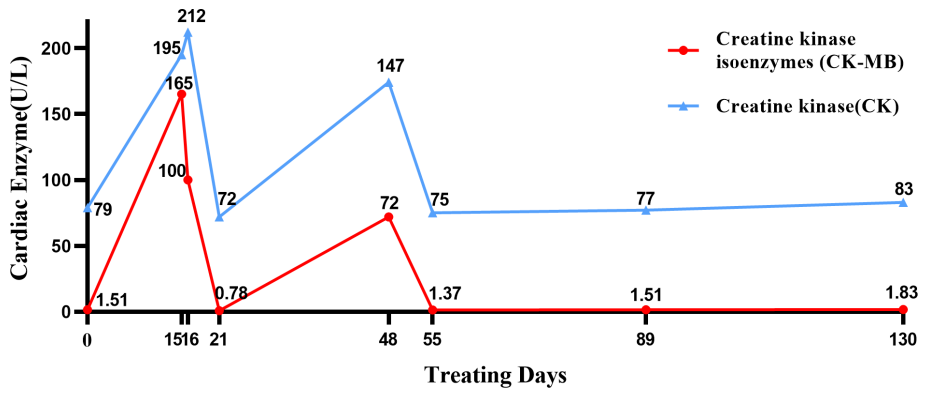

FIGURE 1
Timeline of cardiac enzyme changes. In this figure, we charted the changes in cardiac enzyme levels during the treatment from the day of the patient's first chemotherapy as the starting point for calculation.

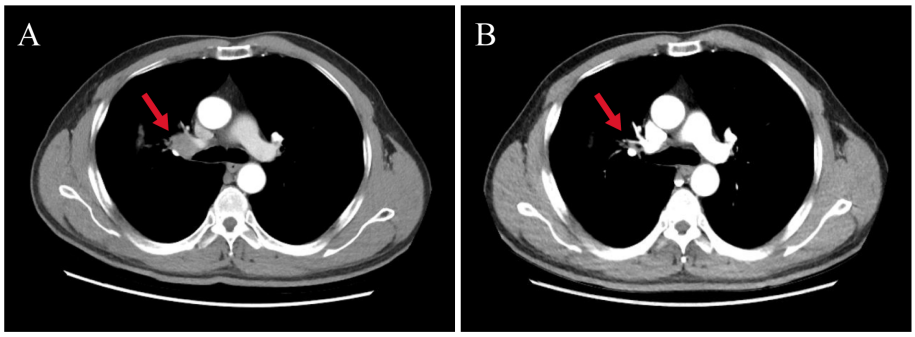

FIGURE 2
Changes in target lesion size during treatment. **(A)** CT scan with chest contrast (before immunotherapy). **(B)** CT scan with chest contrast (after 4 months of sugemalimab treatment). The red arrows in the figure mark the target lesions of enlarged lymph nodes. Chest imaging showed a reduction in the size of the hilar lymph nodes from 2.3 cm to 1.4 cm after four cycles of treatment, with an assessment of PR at the end of treatment.

sugemalimab. During this period, the patient's cardiotoxicity returned to normal while the tumor lesions were well controlled. It suggests that the adverse reaction spectrum may be different between PD-1 inhibitors and PD-L1 inhibitors. A meta-analysis comparing irAEs between PD-1 and PD-L1 inhibitors in multicancer clinical studies included 125 clinical trials with a total of 20,128 patients. The analysis showed that the overall incidence of adverse reactions was lower in PD-L1 inhibitors than in PD-1 inhibitors and the incidence of adverse reactions (>grade 3) was significantly lower in PD-L1 inhibitors than in PD-1 inhibitors (RR: 1.58; 95% CI: 1.00–2.54) (3).

We have considered that it may depend on the different mechanisms of PD-1 inhibitors compared with PD-L1 inhibitors. It has been reported that PD-1 inhibitors simultaneously block the binding of PD-1 on the T-cell surface to PD-L1/2 on the immune cell surface, which increases the risk of potential autoimmune reactions (5). In contrast, PD-L1 inhibitors are able to preserve the immunomodulatory function of the PD-1/PD-L2 pathway and reduce the risk of irAEs. Indeed, the mechanism of ICI-associated cardiotoxicity is not yet clear, and it has been suggested to be possibly associated with infiltration of T cells and macrophages (6). After comparing different PD-L1 inhibitors, we proposed the possible hypothesis that sugemalimab was able to block the binding of PD-L1 to PD-1, resulting in an increased binding of PD-L2 to PD-1, which preserved the immunosuppressive effect of PD-L2. Meanwhile, it also blocks the binding of PD-L1 to CD80, which liberates CD80 and increases the binding of CD80/CTLA-4, exerting immunosuppressive effects and attenuating immune-related toxic reactions. As a unique property of sugemalimab, it activates antibody-dependent cell-mediated phagocytosis (ADCP) *via* binding the Fc segment of the antibody to receptors on the surface of macrophages, inducing further destruction of tumors and resulting a better immunotherapeutic effect. In addition, sugemalimab is a full-length, fully human PD-L1 targeted immunoglobin with lower immunogenicity, which is one of the reasons why it has a lower incidence of irAE (7). Tislelizumab exerts a blocking effect on PD-1 and inhibits both PD-L1 and PD-L2 pathways, causing PD-L1 overexpression. That depletes CD80, making the immunosuppressive effect of the related pathway diminished. At the same time, increased binding of PD-L2 to repulsive guidance molecule B (RGMB) stimulates T-cell activation and may induce autoimmune responses (5, 8). Previous studies have indicated that cardiomyocytes develop immune tolerance mainly by upregulating the expression of PD-L1 to protect cardiomyocytes from immune system attack (9). In a follow-up study of ICI-treated patients, the incidence rates of myocarditis related to PD-1 inhibitors and PD-L1 inhibitors were 0.5% and 2.4%, respectively. Hence, the damage caused by PD-L1 inhibitors to cardiomyocytes may be significant. However, the patient in this case showed less cardiotoxicity after receiving sugemalimab than before and did not develop severe myocardial damage. This result was different from the previous data but was consistent with results obtained from some clinical trials. In a large meta-analysis including 19,217 patients, the incidence of cardiac adverse events was shown to be 33% in anti-PD-1 (n = 9136, 4/12) and 12% in anti-PD-L1 (n = 3164, 3/25) (10). Although certain clinical trials have shown that PD-L1 inhibitors have a lower overall incidence of adverse events than PD-1 inhibitors (3), there are limited studies on mitigating irAE by transitioning PD-1 inhibitors to its ligands. Thus, we can only speculate on the benefits of such therapy from the clinical data. In fact, this case is based on the individual specificity of the patient, and whether the conclusion is of broad significance needs to be pondered. Further research data is necessary to substantiate this point of view. Being a newly approved PD-L1 inhibitor, sugemalimab presents an opportunity for further investigation into whether it possesses its distinct cardioprotective mechanism. However, we need to emphasize that it has no effect on the antitumor effect of the drug after changing the type of ICIs. Immunotherapy is a double-edged sword, and it will be the major challenge to figure out how to use the variations in mechanisms between different types of ICIs to enhance immunotherapy treatment regimens for cancer patients.

In summary, the incidence of cardiotoxicity is <1% in the irAE spectrum (11), including cardiomyopathy (mainly myocarditis), pericardial effusion, arrhythmias, acute coronary syndrome, and heart failure (12). According to previous clinical trials and

retrospective studies, the mortality rate of myocarditis was as high as 39.7%–50% (10, 13). The management of ICI-related cardiotoxicity in the latest domestic and international guidelines recommends corticosteroids as the first-line immunosuppressive drug for ICI-related myocarditis (9), and it is based on AE grading and AE recovery to decide whether to restart the original regimen of immunotherapy. Unfortunately, the lack of recommendations for replacing a different type of ICIs has deprived a number of opportunities for immunotherapy. We anticipate that the paradigm of continuing treatment with PD-L1 inhibitors rather than PD-1 inhibitors after cardiotoxicity develops will provide the patients additional alternatives.

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. Written informed consent was obtained from the participant/patient(s) for thepublication of this case report.

Author contributions

TW and YL collected of data and drafted of the manuscript. CZ and XC revised the manuscript critically for important intellectual content and gave final approval of the manuscript submitted.

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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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Analysis of 12 cases of antineoplastic agents-induced interstitial lung disease

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Objective: To summarize the situation of antineoplastic agents-induced interstitial lung diseases (ILD), provide reference for strengthening clinical management of druginduced interstitial lung diseases (DILD).

Methods: We retrospectively investigated the medical records of 12 patients with antineoplastic agents-induced ILD in a hospital between January and December 2020. Data collected included patients' characteristic (gender, age, ECOG PS score, smoking history, primary tumor, concurrent diseases or complications.) and treatment conditions (DILD-causing drugs, clinical symptoms, chest CT, DILD treatment drugs, onset cycle, onset time, severity of DILD, DILD course and prognosis.).

Results: The median age of 12 DILD cases was 68%, 66.67% of the patients were male, lung cancer accounted for 58.33% (7/12). DILD was induced by cytotoxicity drugs, targeted drugs and immune checkpoint inhibitors (ICIs), of which ICIs accounted for 66.67% (8/12). Scattered patchy, cord-like, grid-like or flocculent shadows were observed on chest CT, mainly under the pleura of lungs. Once DILD occurs, the suspected antineoplastic agents were stopped and glucocorticoid was given, among which 83.33% (10/12) patients were treated with antibiotics. Finally, 16.67% (2/12) were cured, 33.33% (4/12) were improved, 16.67% (2/12) were not cured and 33.33% (4/12) were dead.

Conclusion: Antineoplastic agents-induced ILD is mostly found in elderly male lung cancer patients with smoking history. The clinical symptoms of DILD are diverse and lack of specificity. ICIs-ILD has the characteristic of high incidence and poor prognosis compared with other antineoplastic agents. Comprehensive evaluation before medication, regular review, early and adequate glucocorticoid shock therapy after onset can improve the prognosis of DILD patients.

KEYWORDS

antineoplastic agents, drug-induced interstitial lung diseases, immune checkpoint inhibitors, targeted drugs, glucocorticoid

1 Introduction

Interstitial lung disease (ILD) is a group of heterogeneous diseases caused by multiple etiologies, with interstitial cell proliferation, interstitial matrix hyperplasia and chronic inflammatory cell infiltration as the main pathological changes. Clinically, it is mainly manifested as progressive dyspnea, restrictive ventilation dysfunction with reduced

dispersion function and hypoxemia. Imaging diffuse or multifocal distributed lesions of both lungs, and eventually develop into diffuse pulmonary fibrosis and honeycomb lung (Meyer, 2014; Conte et al., 2022). The American Thoracic Society (ATS) and the European Respiratory Society (ERS) classified ILD into four categories based on etiological, clinical, and pathological characteristics: 1) ILD of known cause; 2) Idiopathic interstitial pneumonia; 3) granulomatous ILD; 4) Other rare ILD, of which known causes of ILD include drug-related. Drug factors in the United States account for 1.9%–3.5% of all ILD (Distefano et al., 2020), while the incidence of DILD in China is underestimated. At present, hundreds of drugs have been known to cause DILD, including anti-tumor drugs, anti-microbial drugs and anti-vascular drugs, etc. In this study, we retrospectively analyzed the medication of ILD caused by anti-tumor drugs in our hospital in 2020, providing a reference for strengthening the management of ILD caused by anti-tumor drugs in clinic.

2 Materials and Methods

2.1 Data source

We used rational drug use system to extract the medical records of patients who received hormone therapy in the Department of Oncology of our hospital from January to December 2020, we consulted medical records through the HIS system, basic information of patients(including gender, age, Eastern Cooperative Oncology Group performance status (ECOG PS) score, smoking history, primary disease, concurrent diseases or complications) were collected, and the treatments of patients (including DILD-causing drugs, onset cycle, onset time, severity of DILD, clinical symptoms, chest CT, DILD treatment agents, DILD course and prognosis) were summarized.

2.2 Criteria for inclusion and exclusion

The diagnosis of ILD caused by antineoplastic agents is suspected in the presence of exposure to a drug known to cause lung toxicity and after exclusion of alternative causes of ILD. Diagnostic criteria are as follows: 1) recent use of antineoplastic agents; 2) clinical manifestations, imaging or pathological features suggest ILD; 3) according to the ADR correlation evaluation (National Center for ADR Monitoring, China, 2017), the time sequence of drug exposure and ILD is reasonable, the reaction stops or improves after drug withdrawal, the reaction reappears after the drug is re-administered, if there are literatures supporting that it is ADR of this drug, the evaluation is “define”; if there is a combination of drugs, but it is almost to exclude the ADR caused by those, the evaluation is “probable”; if the progressive factors of primary diseases cannot be excluded, the evaluation is “possible”; 4) antibiotic treatment is ineffective, symptoms can be relieved and shadows disappear or weaken after hormone treatment; 5) hematological test and bacterial culture were negative. Inclusion criteria: 1) meet the above three rating levels; 2) perform high-resolution computed tomography (HRCT) examination of the lungs; 3) complete clinical data.

TABLE 1 Characteristics of patients with DILD.

Characteristics	N	%
Sex		
Male	8	66.67
Female	4	33.33
Age (years)		
<60	2	16.67
≥60	10	83.33
ECOG PS		
≤1	5	41.67
>1	7	58.33
Smoking history		
Ex/current	7	58.33
Never	5	41.67
Primary tumor		
Lung cancer	7	58.33
Esophageal cancer	3	25.00
B-cell lymphoma	1	8.33
Retroperitoneal leiomyosarcoma	1	8.33
Concurrent diseases/Complications		
Yes	9	75.00
No	3	25.00

Exclusion criteria: 1) previous radiotherapy;2) basic pulmonary diseases (such as chronic obstructive pulmonary disease, pulmonary fibrosis, ILD); 3) infection, heart failure, pulmonary embolism and other diseases leading to dyspnea; 4) alcoholism or mental illness.

2.3 Data processing

Descriptive statistical analysis of data using Microsoft Excel 2019.

2.4 Ethics statement

The studies involving human participants were reviewed and approved by the Institutional Review Board of First People’s Hospital of Lianyungang. The patients provided their written informed consent to participate in this study.

3 Results

3.1 Characteristics of patients

Through the rational drug use system, a total of 1043 patients who used glucocorticoids in the department of oncology from January to December 2020 were extracted, there were 854 cases remain after duplicate removal, through inclusion and exclusion criteria screening, 12 cases meeting the criteria were selected. The basic characteristics of patients are shown in Table 1. The median

age was 68 years (range: 23–78 years), and the ratio of male to female was 2:1, 7 (58.33%) had smoking history, and 9 (75.00%) had other underlying diseases. Lung cancer accounted for more than half of the primary tumors (58.33%), followed by esophageal cancer (25.00%).

3.2 Treatment of patients

Treatment of DILD patients is summarized and shown in Table 2, the specific descriptions are as follows in combination with Table 2:

1. Drug suspected of inducing DILD: DILD-inducing drugs involved 8 cases of ICIs(66.67%), including Pembrolizumab Camrelizumab and Sintilimab,which were programmed death receptor-1(PD-1)inhibitors, 3 cases of targeted drugs and 1 case of cytotoxic drugs.
2. Cycle to onset: The onset cycle was the cycles of using DILD-inducing drugs, the fluctuation range was 1–8, and the median was 2.5. The ICIs-ILD most often occurred after the first cycle of treatment. The onset time of cases 2 and 3 was 4 months and 2 years after treatment respectively, who took small molecule targeted drugs orally.
3. Time to onset: The time between the initial of DILD-related symptoms and the last medication was recorded as the time to onset. The median time was 16.5 days, ranging from 1 day to 63 days.
4. The severity of DILD: The severity of DILD was determined by the Common Terminology Criteria for Adverse Events Version 5.0. All 12 patients with DILD had clinical symptoms, including 6 cases of Grade 2 (50.00 %), 4 cases of Grade 3 (33.33 %) and 2 cases of Grade 4 (16.67 %).
5. Clinical symptoms: The main clinical symptoms of DILD were cough or with a bit of sputum and wheezing, which were aggravated after activity. Cases 8 and 11 had fever as well, up to 39.2°C.
6. Imaging performance:All the 12 patients underwent chest HRCT examination, patchy, streak-like, grid-like and flocculent shadows scattered, multiple or diffusely distributed in both lungs, especially in the subpleural area. Chest HRCT of case 1 and case 2 were shown in Figure 1.
7. Treatments: All DILD patients were treated with glucocorticoid, while symptomatic treatments such as oxygen inhalation, relieving cough, relieving asthma and eliminating phlegm, and correcting acid-base balance were given at the same time. Different types of glucocorticoids are involved in the treatment, all hormone doses were converted to methylprednisolone equivalent doses according to the ratio of methylprednisolone : hydrocortisone : hydrocortisone =4:5:20. Finally, the dose of methylprednisolone was ranging from 40 $mg{\cdot}d^{-1}$ to 160 $mg{\cdot}d^{-1}$, with a median dose of 80 $mg{\cdot}d^{-1}$. 10 patients were given antibiotics concurrently, mainly β-lactamase inhibitor compound (7/10).
8. Course of DILD:The course of DILD was the duration of hormone therapy,with a fluctuation range of 10–103 days, and the median was 35.5 days.
9. Prognosis:After treatment, 16.67% (2/12) were cured, 33.33% (4/12) were improved, 16.67% (2/12) were not cured and 33.33% (4/12) were dead among patients with DILD. Due to long-term application of high-dose glucocorticoid,case 4 had secondary diabetes, osteoporosis and fungal infection.

4 Discussion

4.1 Mechanism of drug-induced interstitial lung diseases induced by antineoplastic agents

Skeoch et al. (2018) reviewed 1694 literatures about ILD and found that antineoplastic agents accounted for 23–51% of all DILD,with the characteristic of low incidence and high mortality. The DILD-induced drugs in this research involved a total of 7, and all the drug instructions indicated the possibility of ILD. The pathogenesis of DILD is poorly understood, but several mechan isms show that ILD induced by cytotoxic drugs may be a direct damage to alveolar epithelial or capillary endothelium cells, or an indirect damage caused by the recruitment of cytokines or inflammatory factor (Ryrfeldt, 2000). Alveolar type II epithelial cells express epidermal growth factor receptor (EGFR), which take part in the repair of alveolar wall. Targeted drugs inhibit not only the growth of tumor, but also the growth of tracheal epithelial cells and the repair of injury, which aggravates lung damage. In addition, targeted drugs may cause damage of alveolar and bronchial epithelial, leading to chronic inflammation, both of which stimulate fibroblast migration, proliferation, and production of extracellular matrix, thereby causing pulmonary fibrosis (Matsuno, 2012). Rituximab-ILD may be caused by the fact that it binds to and kills B cells, resulting in the release of TNF-α IFN-α IL-6 IL-8 and other cytokines by T lymphocytes (Lands, 2010), it may also be caused by a type III hypersensitivity reaction triggered due to its immunogenicity (Lioté et al., 2010). The development of ICIs-ILD may involve dysregulation of immune effector molecules and T cells in the pulmonary interstitium, leading to subsequent inflammatory responses (Delaunay et al., 2017).

4.2 Risk factors for drug-induced interstitial lung diseases

The occurrence and development of ILD induced by antineoplastic agents is still unpredictable, which may be related to many factors, including drug factors and non- drug factors. Drug factors: ①type of drugs; it has been reported that patients receiving targeted and ICIs therapy have a higher incidence of DILD and a worse prognosis than cytotoxic drugs (Nishino et al., 2017), and the incidence of ILD caused by PD-1 inhibitors is higher than that caused by programmed death ligand-1 (PD-L1) inhibitor. ②drug interaction; concomitant use of two or more drugs with pulmonary toxicity is associated with an increased risk of DILD. Non-drug factors: ①Age; the elderly are more likely to have serious adverse reactions due to reduced renal excretion function and reduced liver blood flow, as well as changes in overall metabolic function. ②Sex; Kaku et al. (2022) found that male sex was associated with a higher risk of severe DILD-related death. ③Smoking history; the

TABLE 2 Treatment of patients with DILD.

NO.	Drug suspected of inducing DILD		Onset cycle	Onset time (d)	ADR grade	Clinical symptoms	HRCT	Treatments		DILD course (d)	Prognosis
								Antibiotics	Glucocorticoid (MP dose mg·d^{-1})		
1	Cytotoxic drugs	nab-PTX	2	33	G2	cough with sputum	diffuse grid and patchy shadows in both lungs	piperacillin-tazobactam	80	10	not cured
2	Targeted drugs	Osimertinib	2 years	/	G3	cough and wheezing, worsening after activity	flake and patchy shadows in both lungs	moxifloxacin+ linezolid	80	103	dead
3	Targeted drugs	Crizotinib	4 months	/	G2	cough with a little sputum	multiple flocculent blurs in both lungs	piperacillin-sulbactam	40	16	improved
4	Targeted drugs	Rituximab	2	7	G3	cough and wheezing	multiple patchy shadows in subpleural lung	biapenem	160	69	dead
5	ICIs	Pembrolizumab	1	1	G4	worsening wheezing with chest tightness	scattered flocculent shadows in both lungs	cefoperazone-sulbactam	160	26	dead
6	ICIs	Pembrolizumab	1	1	G2	wheezing	small patchy shadows in both lungs	——	40	36	cured
7	ICIs	Pembrolizumab	8	17	G4	cough with sputum, worsening after activity	multiple patchy density increase in both lungs	cefotaxime-sulbactam	160	64	dead
8	ICIs	Camrelizumab	3	63	G3	worsening cough with wheezing and low-grade fever	patchy blurred shadows in both lungs	cefotaxime-sulbactam	120	47	improved
9	ICIs	Camrelizumab	1	16	G2	cough with sputum	patchy shadows in right lung	cefotaxime-sulbactam	40	10	improved
10	ICIs	Camrelizumab	4	18	G3	cough with sputum	grid and strip shadows in subpleural lung	piperacillin-sulbactam	80	27	not cured
11	ICIs	Sintilimab	1	4	G2	cough and wheezing with high fever	spotted blurred shadows in both lungs	biapenem + vancomycin	120	50	cured
12	ICIs	Sintilimab	4	38	G2	cough with sputum	grid shadows in both lower lungs	——	40	35	improved

ICIs: Immune checkpoint inhibitors, nab-PTX: albumin-bound paclitaxel, MP: Methylprednisolone, /means not applicable, ——means no antibiotics used.

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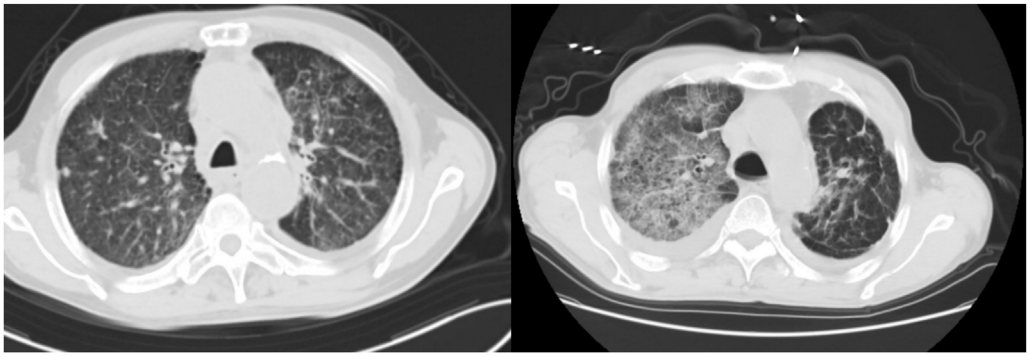

FIGURE 1
Chest HRCT of case 1 and case 2.

risk of DILD of patients who smoked previously is higher than those not (Ando et al., 2006). ④Lung underlying diseases; it has been reported that approximately 20%–24% of patients with preexisting ILD will develop DILD (Isobe et al., 2010). ⑤Radiotherapy; radiotherapy destroys DNA damage and repair proteins involved in the repair of lung injury, especially when combined with chemoradiation, which greatly increases the risk of lung toxicity. ⑥Type of cancers; many lung cancer patients have coexisting ILD, and these patients have a high risk of developing DILD (Raghu et al., 2004), squamous cell carcinoma was also identified as a significant risk factor in the univariate analysis (Sakurada et al., 2015). Among the 12 patients with DILD in this research, patients over 60 years old accounted for 83.33%, 7 patients with lung cancer, the ratio of male to female was 2:1, and the most of the male patients had a history of smoking, all of 8 patients with ICIs- ILD were induced by PD-1 inhibitors, which was consistent with results of studies on risk factors for DILD. The case 11 had a history of psoriasis, and ILD occurred after the first cycle of immunotherapy. Therefore, it is essentital to choose drugs with low pulmonary toxicity in patients with advanced age and smoking history, ICIs is especially prudent for patients with autoimmune diseases.

4.3 Onset time and clinical symptoms of drug-induced interstitial lung diseases

The time to onset of ILD after initiation of antineoplastic agents ranges from a few days to several years, and the pulmonary toxicities often present with relatively non- specific features. Symptoms might include dyspnoea, hypoxia, cough, chest discomfort, or, less commonly, fever, a few patients with DILD have no clinical symptoms just imaging abnormalities (Rashdan et al., 2018), ground-glass nodules or patchy nodular infiltration in the lower lobes of both lungs was detected in the CT findings of these patients. It was reported that the median onset time of ICIs-ILD is 2.8 months (Naidoo et al., 2017), ILD induced by Osimertinib occurs after 3 months while nab-PTX- induced ILD occurs from weeks to months after treatment (Tan et al., 2020). In summary, ILD occurs later than other adverse drug reactions, while combination therapy occurs earlier. The clinical manifestations of the 12 patients with DILD in this research were different, including cough (83.33%), sputum cough (50.00%), wheezing (58.30%), chest tightness (8.33%) and fever (16.67%), while HRCT findings of new patchy or cord-like blurred shadows compared with before. The earliest onset time of DILD was one day after the first cycle of Pembrolizumab, and the latest was 2 years after the medication of Osimertinib. The onset time of ILD induced by the same drug in disparate patients is also different. Therefore, when patients use drugs with pulmonary toxicity, it is necessary to pay attention to new discomfort and perform chest CT routinely, so as to achieve early detection and early treatment, and reduce the adverse effects of DILD on patients.

4.4 Treatment and prognosis of drug-induced interstitial lung diseases

The goal of ILD treatment is to suppress the inflammatory response, promote exudation absorption, prevent pulmonary interstitial fibrosis, and protect cardiopulmonary function. Refer to the diagnosis and treatment guidelines and expert consensus of ILD induced by antineoplastic drugs at home and abroad (Zhang et al., 2021; Chinese Society of Clinical Oncology, 2022; National Comprehensive Cancer Network, 2023), the treatment strategies are formulated as follows: 1) Discontinuation of (and avoidance of further exposure to) the culprit drug is themainstay of treatment; 2) Patients with confirmed or suspected to have DILD are generally treated with glucocorticoid (methylprednisolone 0.5–4.0 mg/(kg·d) or equivalent according to severity grade), and plan a slow glucocorticoid taper over ≥ 6 weeks. It is also important to supplement calcium and vitamin D, monitor levels of oxygen saturation, blood pressure, blood sugar, and prevent gastrointestinal bleeding; 3) Anti-infective therapy is recommended if co-infection cannot be ruled out (sensitive anti-infective drugs are selected on demand or based on microbiological findings); 4) Oxygen therapy: It is recommended that ILD patients with resting hypoxemia receive long-term oxygen therapy for more than 15 h/d according to the indications for oxygen therapy in chronic obstructive pulmonary disease. For ICIs- ILD patients with Grade3-4,if no improvement is observed after 48 hours of treatment, consider additional immunosuppression with any of the following agents: infliximab, IVIG, or mycophenolate mofetil. 12 patients with

DILD in this research discontinued suspect drugs and started glucocorticoid immediately, 10 of them in combination with antibiotics. The patients who were treated with adequate hormone according to the principle of treatment accounted for 66.67% (8/12), among which 50% (4/8) died, while the patients with low- dose hormone improved or cured eventually. This means that the prognosis of patients with DILD in this research is not directly related to the dose of hormone, but may be related to the severity of DILD and the sensitivity of the body to glucocorticoid. The prognosis of patients with severe disease or low response to hormone is poor. Two of the four death cases in this research were caused by Pembrolizumab, ICIs were used as second- or third-line treatment while cytotoxic agents were first-line in lung cancer, it may be a reason for the worse prognosis of ICIs-ILD than ILD induced by cytotoxic agents.

4.5 Rechallenge with causative drugs

All patients with DILD in this research discontinued suspected drugs permanently. Up to now, there is no international consensus on the risks and benefits of rechallenge after ILD induced by antineoplastic agents. Refer to NCCN guideline Version1. 2023-management of immunotherapy-related toxicities (National Comprehensive Cancer Network, 2023) and FUSCC criteria for the management of targeted drug-induced interstitial lung disease in solid tumors (Zhang et al., 2021),for ILD induced by targeted drugs, Grade4 is recommended to be discontinued permanently, while Grade2-3 is recommended to be reduced by one therapeutic dose level after recovery; it is recommended to discontinue ICIs for life for Grade3-4 ICIs-ILD, and ICIs can be rechallenged after recovery from Grade2 ICIs-ILD. Gu et al. (2021) had reported a case of successful Osimertinib rechallenge after recovery from Osimertinib-induced ILD in a patient with EGFR-mutant non-small cell lung cancer, which experienced Grade2 ILD and recovered after glucocorticoid therapy for 13 days. The patient received Osimertinib treatment (80 mg qd) again, and oral prednisone was given concurrently, there was no disease progression or ILD recurrence within more than 16 months of treatment. Clinically, if the drug is essential and could not be replaced, rechallenge should always be discussed with a multi-disciplinary team.

5 Conclusion

DILD is a rare adverse reaction of antineoplastic drugs. Due to the lack of specificity of clinical manifestations and different onset time, patients often miss the best treatment time and affect the survival time. Accurate identification of risk factors can help screen high-risk patients before treatment with relevant drugs. During the treatment period, it is necessary to closely monitor the changes of the disease condition, especially the respiratory function and chest CT. Meanwhile, the medication education for the patients should be strengthened to improve the cognitive level of the patients. Once the disease condition changes, early identification, early diagnosis and timely treatment can avoid serious consequences. It is equally important to prevent the complications such as osteoporosis, gastrointestinal bleeding, and fungal infection during the treatment of DILD. However, the decision on whether to rechallenge the same causative drug after remission of DILD requires careful consideration of risks and benefits as well as the availability of alternative treatments.

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 humans were approved by the Institutional Review Board of First People's Hospital of Lianyungang. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements. 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

Concept and design of the study by XL, Y-LG, Z-XS, and YS Data collection and management of the study by XL, X-CL, and YS Statistical analysis by XL, Y-LG and X-CL. Drafting of the manuscript by XL and Z-XS. 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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CU06-1004 as a promising strategy to improve anti-cancer drug efficacy by preventing vascular leaky syndrome

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Background: Interleukin-2 (IL-2) is the first cancer therapeutic agent with an immunomodulatory function. Although it has been experimentally proven to be effective against metastatic renal cell carcinoma and metastatic melanoma, the clinical application of high-dose IL-2 (HDIL-2) has been limited because of its short half-life and severe side effects, such as vascular leakage syndrome (VLS) or capillary leaky syndrome (CLS). However, methods for overcoming this issue have not yet been identified.

Methods: We discovered CU06-1004, an endothelial dysfunction blocker, through a previous study, and co-treated with IL-2 immunotherapy to confirm its inhibitory effect on HDIL-2-induced endothelial permeability. CU06-1004 was co-administered with HDIL-2 for 4 days in an *in vivo* mouse model. After drug injection, the mice were sacrificed, and Evans blue staining was performed.

Results: *In vitro*, HDIL-2 treatment decreased HUVEC stability, which was rescued by co-treatment with CU06-1004. In our mouse model, co-administration of CU06-1004 and HDIL-2 prevented HDIL-2-induced vascular leakage by normalizing endothelial cells. Notably, the HDIL-2 and CU06-1004 combination therapy considerably reduced tumor growth in the B16F10 melanoma mouse model.

Conclusion: Our data suggest that CU06-1004 acts as a potential anticancer drug candidate, not only by preventing HDIL-2-induced VLS but also by enhancing the anticancer effects of HDIL-2 immunotherapy.

KEYWORDS

IL-2 immunotherapy, permeability, vascular leaky syndrome (VLS), drug side effect, endothelial dysfunction blocker

1 Introduction

Cancer immunotherapy has indeed revolutionized anti-cancer drugs, and IL-2 is a key immunotherapeutic agent used to stimulate the immune system to attack cancer cells (Farkona et al., 2016; Zhao et al., 2019; Zhang and Zhang, 2020). It exhibits pleiotropic effects on the immune system, particularly its ability to promote the development of white blood

Abbreviations: 1004, CU06-1004; IL-2, Interleukin 2; VLS, Vascular leaky syndrome; CLS, Capillary leaky syndrome; AJ, adherent junction.

cells and release chemicals that attract cancer-killing immune cells; these effects make it a valuable tool in the fight against cancer (Galli et al., 2020; Pena-Romero and Orenes-Pinero, 2022). High-dose IL-2 (HDIL-2) was approved by the FDA for the treatment of metastatic renal cell carcinoma in 1992 and of metastatic melanoma in 1998 (Payne et al., 2014; Alva et al., 2016).

However, its use was limited due to its short half-life and serious side effects (Skrombolas and Frelinger, 2014; Sun et al., 2019; Merchant et al., 2022). Similar to the commonly known side effects of anticancer drugs, IL-2 therapy causes a flu-like syndrome, fever, nausea, vomiting, and asthenia (Altun and Sonkaya, 2018; Mortara et al., 2018). However, the major side effect of HDIL-2, used for cancer regression, is vascular leaky syndrome (VLS) and capillary leaky syndrome (CLS) (Baluna and Vitetta, 1997; Sivakumar et al., 2013; Kim et al., 2014). Severe VLS is caused by HDIL-2 treatment, which increases vascular permeability and decreases microcirculatory perfusion (Moreno et al., 2006; Guan et al., 2012; Sivakumar et al., 2013). Ultimately, it causes extensive fluid retention in multiple organs, such as the lungs, liver, and heart, and can lead to pulmonary edema, liver cell damage, and cardiovascular failure (Krieg et al., 2010; Chen et al., 2018).

To overcome this effect, we administered combination therapy with CU06-1004, a previously known endothelial dysfunction blocker (Park et al., 2020; Bae and Kwon, 2022; Zhang et al., 2022). CU06-1004 sustains vascular stabilization and strengthens the endothelial barrier (Park et al., 2020). In addition, CU06-1004 inhibits vascular leakage by forming cortical actin rings via cAMP/Rac/cortactin (Kim D. Y. et al., 2020). It leads to the regulation of various factors such as vascular endothelial growth factor (VEGF), histamine, and thrombin (Kim Y. S. et al., 2020; Park et al., 2020). In a previous study, CU06-1004 reduced IL-1β-induced endothelial permeability and NF-κB activation, neurological deficits, cerebral infarction, and glial activation in an ischemic stroke mouse model (Kim D. Y. et al., 2020). The therapeutic effects of CU06-1004 have been demonstrated in various disease models, such as cancer, stroke, and diabetic retinopathy. Our cancer study showed that CU06-1004 induced tumor vessel normalization by enhancing junction proteins, pericytes, and smooth muscle actin and overcame tumor progression and treatment resistance (Park et al., 2020).

Notably, co-administration of CU06-1004 and IL-2 has shown potential in overcoming severe vascular leaky syndrome induced by HDIL-2 therapy. We overcame the decreased viability and increased permeability of endothelial cells induced by IL-2 in an *in vitro* HUVEC model by co-injection with CU06-1004. In addition, we established IL-2-induced side effects in an *in vivo* model and confirmed the reduction of side effects through combined administration. Additionally, HDIL-2 produced tumor suppression and reduced side effects in the B16F10 tumor-bearing mouse model. This suggests that CU06-1004 could serve as a potential anticancer drug candidate, not only by preventing HDIL-2-induced VLS but also by enhancing the anticancer effects of HDIL-2 immunotherapy.

2 Materials and methods

2.1 Cell lines and culture

Human umbilical vein endothelial cells (HUVECs) were purchased from Lonza (Basel, Switzerland). HUVECs were cultured in plates coated with 2% gelatin (Sigma-Aldrich) and endothelial cell basal medium-2 (Lonza) supplemented with EGM SingleQuots (Lonza) at 37°C in 5% CO_2. B16F10 murine melanoma cells (kindly gifted by Prof. Sang-Jun Ha; Yonsei University, Seoul, Korea) were cultured in complete Dulbecco's modified Eagle's medium (DMEM; Hyclone; SH30022.01) supplemented with 10% fetal bovine serum (FBS; GE Healthcare UK Ltd.) and 1% penicillin/streptomycin (Gibco Laboratories) at 37°C in 5% CO_2 incubator in a humidified atmosphere.

2.2 Mice

Male C57BL/6 mice, aged 6–7 weeks, were purchased from DBL Korea under semi-SPF conditions. All experiments were approved by the committee of Yonsei University (IACUC-A-202104-1252-01).

2.3 Drugs

Recombinant IL-2 immunotherapy (Recombinant human IL-2; 200-20) was purchased from Peprotech, Korea. CU06-1004 has been previously reported (Maharjan et al., 2013; Lee et al., 2014). To synthesize CU06-1004, a tetrahydropyran analog was prepared by reacting dihydropyran and pregnenolone in *p*-toluenesulfonic acid. After Wittig olefination with 4-(carboxybutyl) triphenylphosphonium bromide, the acid moiety was methylated using trimethylsilyl diazomethane. CU06-1004 was synthesized via tetrahydropyran deprotection and subsequent glycosidation with 4, 6-di-*O*-acetyl-2, 3-didieoxyhex-2-enopyran in the presence of an acid.

2.4 IL-2 and CU06-1004 treatment

Recombinant IL-2 drug was intraperitoneally (i.p.) injected with 75,000 U of three times a day for 3 consecutive days. On day 4, the mice received one injection. After 2 h, the mice were sacrificed. CU06-1004 was dissolved in 100 μL of olive oil (Sigma-Aldrich, St. Louis, MO), and a dose of 10 mg/kg was administrated by using oral gavage daily for the same duration as the IL-2 drug.

2.5 *In Vitro* cell cytotoxicity

Cell viability and proliferation were compared by 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium-bromide (MTT) assay. HUVEC were seeded in 24-well plates (2 X 10^4 cells/well). After treatment with CU06-1004 and IL-2, cells were maintained for 24 h in media containing 0.2% FBS. MTT (0.5 mg/mL) was added to each well, and cells were incubated at 37°C for 3 h. The supernatant was removed, and 200 μL DMSO + isopropyl alcohol was added to dissolve the formazan product. Absorbance, which is proportional to the number of living cells and proliferation rate, was measured at 540 nm on a microplate reader (FLUOstar Omega, BMG LABTECH). Data represent four independent experiments.

2.6 Immunofluorescence staining of human umbilical vein endothelial cells (HUVECs)

To examine vascular permeability, HUVEC were fixed in 4% paraformaldehyde for 10 min and permeabilized with 0.1% Triton X-100 in PBS for 15 min at room temperature. The cells were incubated with primary antibodies against VE-cadherin (1:200; Santa Cruz Biotechnology) at 4°C for 16 h. Cells were then incubated with secondary antibodies conjugated to Alexa Fluor 594 for 1 h at room temperature. Actin filaments were incubated with rhodamine-phalloidin (1:250; Molecular Probes) for 30 min. For nuclear staining, the cells were treated with DAPI (1:1000) for 20 min before mounting. Immunofluorescent images were obtained using a confocal microscope (Carl Zeiss 700, Germany).

2.7 Endothelial cell permeability assay

Human umbilical vein endothelial cells were seeded at a density of 4×10^5 cells/well onto 12-well Transwell semipermeable supports (0.4 μm pore size; Corning) coated with 1% gelatin. HUVEC were cultured in EC basal medium (EBM-2, CC-3156) containing EGM-2-kit (CC-4176) (Lonza Walkersville, Inc., MA, United States) and 10% FBS at 37°C in a 5% CO_2 incubator in a humidified atmosphere. Upon confluence, the cells were starved in serum-depleted medium for 2 h and treated with 100 kilounits/mL IL-2 for 4 h. Endothelial cell permeability was confirmed using fluorescein isothiocyanate (FITC)-dextran fluorescein. FITC-dextran (30 mg/mL; Sigma-Aldrich) was added to the upper chamber and incubated for 30 min. The absorbance was measured at 492 nm (excitation) and 520 nm (emission) using a FLUOstar Omega microplate reader. The transendothelial electrical resistance (TEER) assay was performed using a chopstick electrode (World Precision Instruments STX2) with Millicell ERS-2 volt/Ω m (Millipore, MA, United States) and the results expressed as $\Omega \times cm^2$.

2.8 *In vivo* tumor models

Tumors were subcutaneously implanted into the right flanks of 6- to 7-week-old C57BL/6 mice. Tumor volumes were measured every day according to formula (0.523 x (length x width^2)). The drug was injected approximately 1 week after the tumor was implanted.

2.9 Evans blue staining

To analyze vascular permeability, mice were injected intravenously (i.v.) injected with 1% Evans blue dye (Sigma-Aldrich) diluted in 100 μL saline. Fifteen minutes later, mice were anesthetized with 2.5% avertin (Sigma-Aldrich) via intraperitoneal (i.p.) injection. And mice were perfused with 20 mL PBS, and tissues (Lung, Liver, Hand, Foot) were harvested, and dye was extracted in Formamide (500 μL, Junsei, Tokyo, Japan) overnight at 60°C. Dye concentrations were quantified by measuring absorbance at 620 nm. The content of Evans blue dye was determined by generating a standard curve from dye dilutions.

2.10 Immunofluorescence staining of tumor tissue

Mice were anesthetized with i. p. 2.5% avertin and then perfused with 50 mL PBS or saline via the left ventricle of the heart. Whole tumors were collected, fixed with 4% paraformaldehyde (PFA) for 16 h (h), dehydrated in a 15% sucrose solution, and followed by a 30% sucrose solution until tumors sank to the bottom of the container. Mouse tumor tissues were sectioned 20–30 μm thick using a cryostat (Leica, Wetzlar, Germany). One of every 7 to 10 slices was collected. Sections were stored at −80°C. To examine increased T cells in the tumor, CD8 (Abcam; ab22378; 1:200) was performed at 4°C for 16 h. After washing, slides were incubated with the appropriate Alexa-Flour 488-conjugated secondary antibodies (1:500) at RT for 1 h. Immunofluorescence was imaged using confocal microscopy (Carl Zeiss 700, Germany). Quantification of fluorescence intensity and cell counting was performed using Image J (NIH) or Photoshop version CS6 (Adobe Systems, San Jose, CA).

2.11 Enzyme-linked immunosorbent assay

To analyze cytokine levels between drug-treated groups, protein was collected from each Mouse serum. Quantification was performed with BCA protein reagent (SMART™ BCA Protein Assay Kit Solution A and B; iNtRON BIOTECHNOLOGY; 21071) and RIPA buffer assay (cOmplete ULTRA Tablets; Roche). IFNγ (Mouse IFN-gamma Quantikine ELISA; R&D Systems; MIF00) was measured by ELISA kit.

2.12 Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). All statistical analyses were performed using GraphPad Prism (version 8; GraphPad Software, La Jolla, CA). The mean difference between groups was also analyzed by one-way ANOVA. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$. ns, not significant.

3 Results

3.1 CU06-1004 alleviates vascular hyper-permeability by preventing the reduction of endothelial cell viability by IL-2

IL-2 immunotherapy causes endothelial dysfunction, eventually inducing VLS. Therefore, we co-injected IL-2 and CU06-1004 to resolve the IL-2-induced side effects in endothelial cells. In previous studies, CU06-1004 has been reported as a blocker of endothelial dysfunction (Kim D. Y. et al., 2020; Kim Y. S. et al., 2020; Park et al., 2020; Bae et al., 2021). We first tested cell viability in the IL-2-alone group and the IL-2- and CU06-1004- combination group in HUVEC. As a result of the MTT assay to compare cell viability, the number of HUVECs was decreased in the IL-2 alone group. However, it was confirmed that the number of HUVECs

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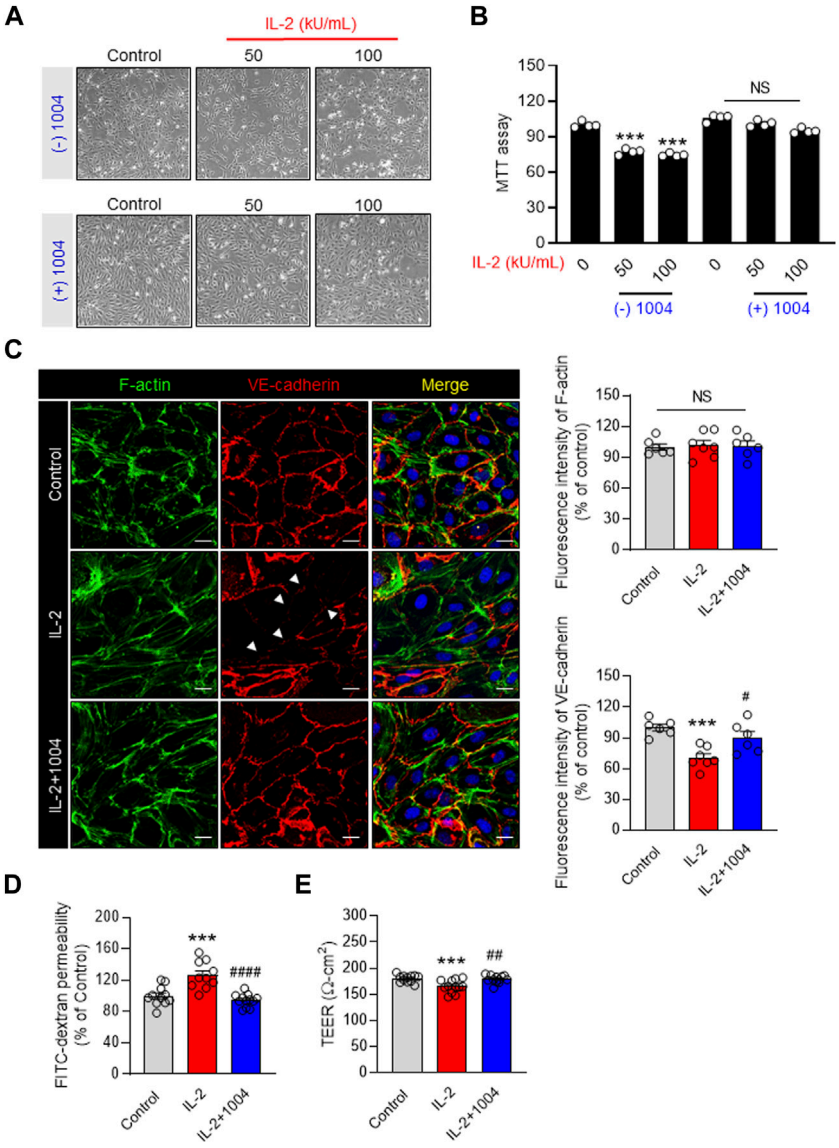

FIGURE 1
EC dysfunction blocker CU06-1004 improves the decrease in IL-2-induced endothelial cell viability and permeability. **(A, B)** HUVEC MTT assay was performed using a 24-well cell culture plate. After treatment with CU06-1004 (10 mpk) and IL-2 (100 kU/mL), the cells were incubated in a medium containing 0.2% FBS for 24 h. Data represent four independent experiments. Statistical analysis by one-way ANOVA with Tukey's multiple comparisons. **(C)** IL-2 and CU06-1004 were administered after HUVEC starvation. Subsequently, the cells were fixed, permeabilized, and stained for adherent junction marker and F-actin. Green, F-actin; Red, VE-cadherin; blue, DAPI staining. $n \geq 3$ independent experiments. **(D)** HUVEC were starved and treated with CU06-1004 and IL-2 for 4 h. Next, FITC-dextran (30 mg/mL; Sigma) was added to the upper chamber and incubated for 30 min. Absorbance was measured at 492 nm (excitation) and 520 nm (emission) using a FLUOstar Omega microplate reader. $n \geq 3$ independent experiments. **(E)** The TEER assay was performed using a chopstick electrode (World Precision Instruments STX2) with Millicell ERS-2 volt/Ω m (Millipore, MA, United States) and given in ohm cm squared. $n \geq 3$ independent experiments. Statistical analysis by one-way ANOVA with Tukey's multiple comparisons. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. ns, not significant. Data are presented as ± SEM.

in the IL-2 and CU06-1004 combination group was increased compared to IL-2 alone (Figures 1A, B). Therefore, we analyzed whether IL-2-induced permeability could be reduced by increasing cell viability by CU06-1004. It has been reported that stimulation with IL-2 disrupts interactions between adhesive junction (AJ) proteins, alters cell morphology and creates gaps between adjacent cells. Therefore, we performed VE-cadherin and F-actin immunostaining in the IL-2 alone and IL-2 and CU06-1004 combination group. At the cell borders, AJ proteins from normal HUVEC formed a linear pattern. Co-administration of CU06-1004 restored the linear pattern that was collapsed by IL-2 alone. Furthermore, in the IL-2 alone group, it was seen that the stress fiber was relatively increased compared to the normal group. However, the combination group with CU06-1004 showed no increase in stress fibers compared to the IL-2 alone group (Figure 1C). Additionally, We measured TEER and FITC-dextran in the HUVEC monolayers to determine endothelial barrier integrity and permeability. TEER decreased, and CU06-1004

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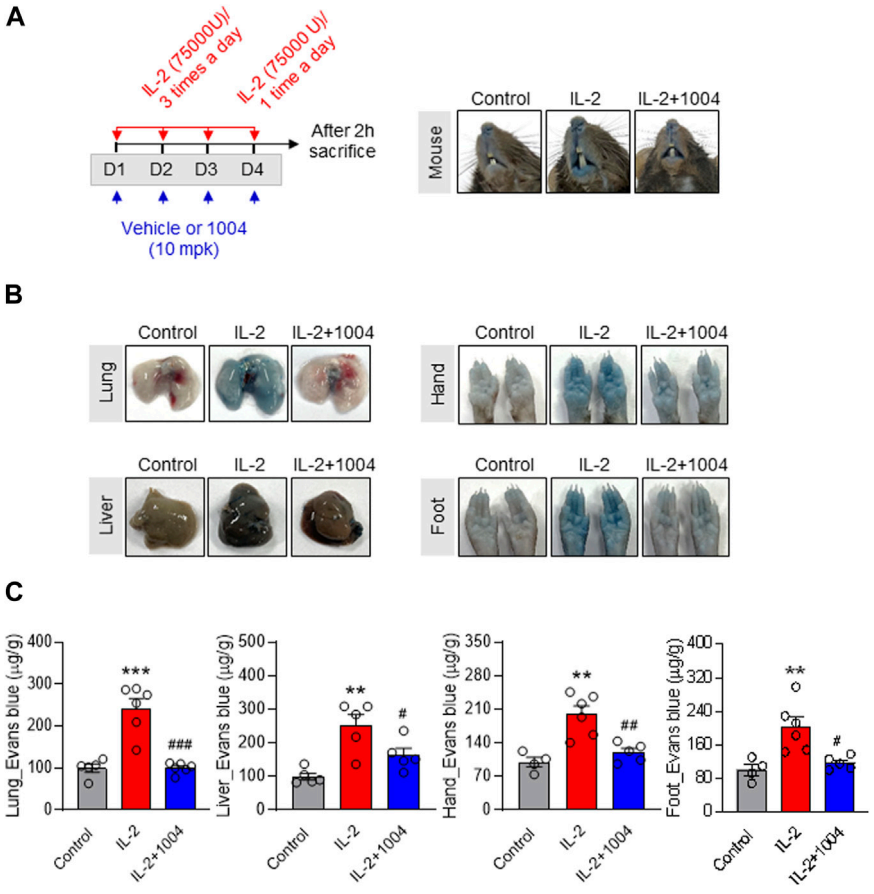

FIGURE 2
CU06-1004 alleviates IL-2-induced vascular leaky syndrome through Evans blue staining in normal mouse model. **(A)** Schematic depicting the schedule of IL-2 and CU06-1004 treatments in normal mice. Treatments were performed under the condition of IL-2-induced vascular leaky syndrome. Groups of four to five mice were injected i. p. with 75,000 U of IL-2 or PBS as a control three times a day for 3 consecutive days. On day 4, the mice received one injection, and 2 h later, they were injected. They were orally injected with 10 mpk (10 mg/kg) CU06-1004 daily. After 2 h, the mice were sacrificed. **(B–C)** Vascular permeability was quantified by i. v. administration of Evans blue dye. After dye administration, the mice were perfused with PBS, tissues were harvested, and dye extracted in formamide overnight. Dye concentrations were quantified by measuring absorbance at 620 nm. The content of Evans blue dye was determined by generating a standard curve from dye dilutions. (B) The representative image shows Evans blue staining in a normal mouse model after drug treatment. **(C)** The representative graph shows the percentages of VLS level by Evans blue staining. n = 4-6 per group. Statistical analysis by one-way ANOVA with Tukey's multiple comparisons. *p < 0.05; **p < 0.01; ***p < 0.001. ns, not significant. Data represent ± SEM.

changed the IL-2-induced hyper-permeability (Figures 1D, E). Ultimately, Our results showed that CU06-1004 suppressed the IL-2-induced negative effects by protecting the viability and reducing the permeability of endothelial cells.

3.2 Treatment of CU06-1004 ameliorates vascular leakage by HDIL-2 immunotherapy *in vivo*

We previously showed that CU06-1004 protects against IL-2-induced endothelial damage. To confirm the reduction in vascular leakage in tissues by CU06-1004, we conducted Evans blue staining in normal mice by injecting the dye into the tail vein and measuring the amount of dye outflowing from the vasculature. IL-2 stimulation significantly induced vascular leakage in the tissues and skin, and IL-2-induced hyperpermeability was reduced owing to leakage blocking by CU06-1004 (Figures 2A–C). This result suggests the possibility of improving the VLS or CLS induced by HDIL-2 through combination therapy with CU06-1004.

3.3 CU06-1004 improves IL-2-induced VLS in the B16F10-bearing mouse model

In a previous result, IL-2-induced vascular leakage in normal mice was observed through Evans blue staining, and it was found that co-administration with CU06-1004 improved this effect. Therefore, in Figure 3 of our study, we aimed to observe the degree of vascular leakage by IL-2 and CU06-1004 in the B16F10-bearing mouse model using Evans blue staining. To compare the degree of vascular leakage between groups, the amount of Evans blue dye was measured in Lung, Liver, Hand, and Foot. The results showed that vascular leakage in the IL-2 alone group was significantly increased compared to the control group, while it was decreased in the combination group with CU06-1004

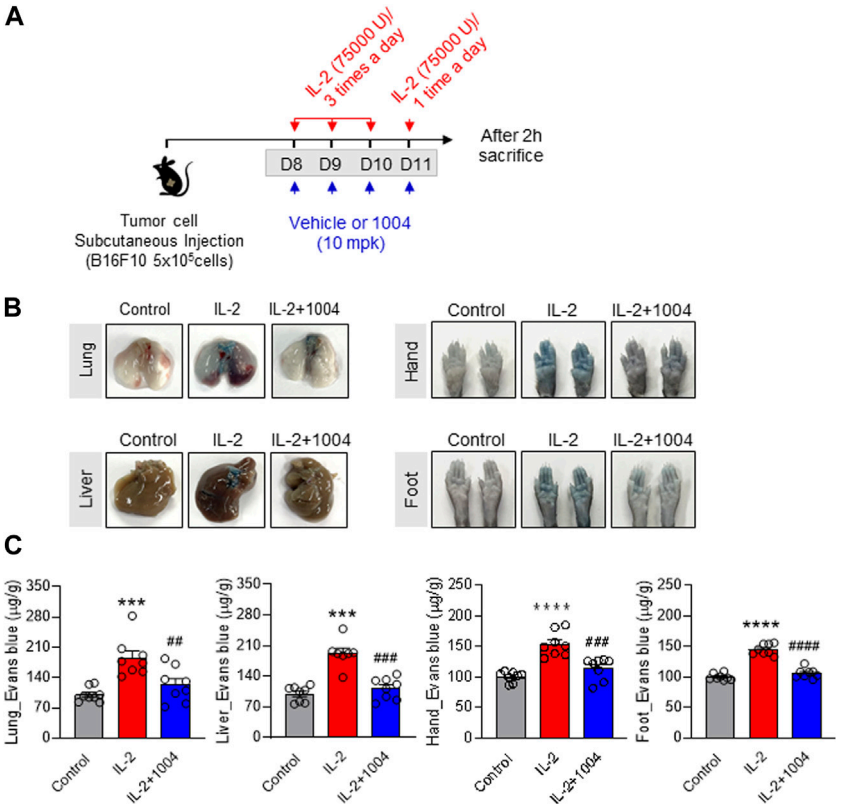

FIGURE 3
CU06-1004 alleviates IL-2-induced vascular leaky syndrome in B16F10 tumor-bearing model. **(A)** Schematic depicting the schedule of combination therapy in a B16F10 tumor-bearing mouse. **(B–C)** Vascular permeability was quantified by i. v. administration of Evans blue dye. After dye administration, mice were perfused with PBS, tissues were harvested, and dye extracted in formamide overnight. Dye concentrations were quantified by measuring absorbance at 620 nm. The content of Evans blue dye was determined by generating a standard curve from dye dilutions. **(B)** The representative image shows Evans blue staining in B16F10 tumor-bearing model after drug treatment. **(C)** The representative graph shows the percentages of VLS level by Evans blue staining. $n = 8$ per group. Statistical analysis by one-way ANOVA with Tukey's multiple comparisons. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. ns, not significant. Data represent ± SEM.

compared to the IL-2 alone group (Figures 3A–C). These results demonstrated that the inhibition of vascular leakage in the tumor-bearing mouse model was the result of CU06-1004 injection, and emphasized that the side effects of IL-2 drugs could be improved by co-administration with CU06-1004.

3.4 Combination therapy maintains the tumor-killing effect in the B16F10-bearing mouse model

Our results to date have demonstrated that CU06-1004 can reduce IL-2-induced vascular leakage in a tumor-bearing mouse model. However, to consider its potential application in cancer patients, it is crucial to prove its tumor suppression effect in combination therapy. To show this, we monitored the size and weight of tumors in three groups: the control group, the IL-2 alone group, and the combination group with CU06-1004 (Figure 4A). Upon analysis, we found that the tumor size and weight in the IL-2 alone group were significantly reduced compared to the control group, indicating the efficacy of IL-2 in tumor suppression. Interestingly, in the combination group with CU06-1004, we also observed a significant reduction in tumor size and weight compared to the control group (Figures 4B,C). This intriguing finding suggests that the co-administration of CU06-1004 with IL-2 not only maintains the tumor suppression effect of IL-2 but also potentially enhances it. The data obtained from this study further supports the therapeutic potential of the combination therapy, indicating that CU06-1004 may complement the anti-cancer efficacy of IL-2 treatment.

3.5 $CD8^+$ T cells are increased in CU06-1004- and IL-2-injected group

Next, in order to identify the number of immune and inflammatory cells in the tumor microenvironment, we conducted immunofluorescence staining of $CD8^+$ T cells with cytotoxic abilities. Interestingly, when the tumor size was reduced by the IL-2 drug, the number of immune cells infiltrated into the tumor was significantly increased. However, there was no significant difference between the IL-2 alone group and the CU06-1004 co-administration group (Figure 5A). Additionally, to compare the cytokine changes caused by the increased $CD8^+$ T cells, we

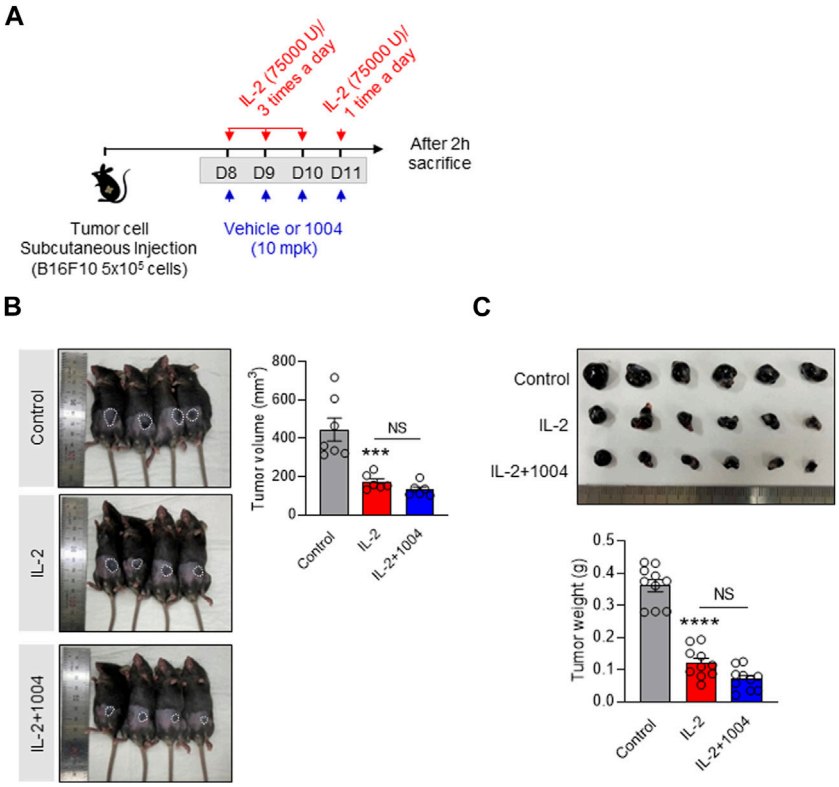

FIGURE 4
Combination of CU06-1004 and IL-2 decreased B16F10 melanoma growth and sustained IL-2 immunotherapy efficacy. **(A)** Schematic depicting the schedule of combination therapy in a B16F10 tumor-bearing mouse. B16F10 tumor cells (5×10^5 cells/mouse) were injected subcutaneously into the right flank of C57BL/6 mice. The tumor-bearing mice were treated with IL-2 and CU06-1004 after tumor inoculation (tumor size <100 mm^3). Groups of four to five mice were injected i. p. with 75,000 U of IL-2 or PBS as a control three times a day for 3 consecutive days. On day 4, the mice received one injection, and 2 h later, they were sacrificed. **(B)** Tumor volume (n = 6-7 per group) and **(C)** weight (n = 10) were measured from each group of mice. Statistical analysis by one-way ANOVA with Tukey's multiple comparisons. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. ns, not significant. Data are presented as ± SEM.

confirmed the expression of IFNγ in mouse serum between the groups by ELISA. As a result, the expression of IFNγ was significantly increased in both IL-2 injected groups. These results were proportional to the increase in the number of $CD8^+$ T cells with cytotoxic capability in tumors (Figure 5B). In summary, tumor size reduction by IL-2 drugs is expected to be related to the number of $CD8^+$ T cells in the tumor and the expression of pro-inflammatory cytokines.

4 Discussion

Recently, human cancer treatment has developed rapidly with the targeting of immune response 'checkpoints' using cytotoxic T lymphocytes (Zhang et al., 2018; Waldman et al., 2020; Raskov et al., 2021). Regulation by T cells has a surprising effect on tumor regression by releasing cellular immune response, enabling long-term treatment (Walsh et al., 2019; Waldman et al., 2020). Nevertheless, according to a recent report, immunotherapies such as IL-2 cause fatal and serious side effects related to endothelial disorders (Li et al., 2017; Mortara et al., 2018). High doses of IL-2 therapeutics are correlated with treatment success, but lower doses of IL-2 reduce side effects and responses (Davar et al., 2017). High doses of IL-2 therapeutics are correlated with treatment success, but lower doses of IL-2 reduce side effects and responses (Rosenberg et al., 1994). Therefore, we co-administered CU06-1004, an endothelial dysfunction blocker, to suppress VLS (CLS) and cytokine 'storm', the most serious side effect of HDIL-2 therapy (Fig.6) (Skrombolas and Frelinger, 2014).

First, we performed an MTT assay in HUVEC to confirm that CU06-1004 recovered IL-2-induced toxicity at the cellular level. Depending on the concentration of IL-2, endothelial cells were separated from the extracellular matrix and intercellular junctions were degraded, leading to pores. However, the co-administration of CU06-1004, which induces endothelial cell stabilization and normalization, inhibited IL-2-induced damage and apoptosis. In addition, previous studies have reported that IL-2 increases endothelial cell permeability, which induces hypoalbuminemia in patients (Xie et al., 2012; Zloza et al., 2014; Soeters et al., 2019). This hypothesis is consistent with the results of our previous permeability assays. To achieve endothelial cell integrity, the cytoskeletal tissue and intercellular junctions, such as the AJ, must be well maintained; however, they have been reported to be dissolved by several permeable factors. In particular, strongly permeable factors, such as HDIL-2, significantly increase actin stress fibers and induce endothelial cell permeability. However, our results demonstrated

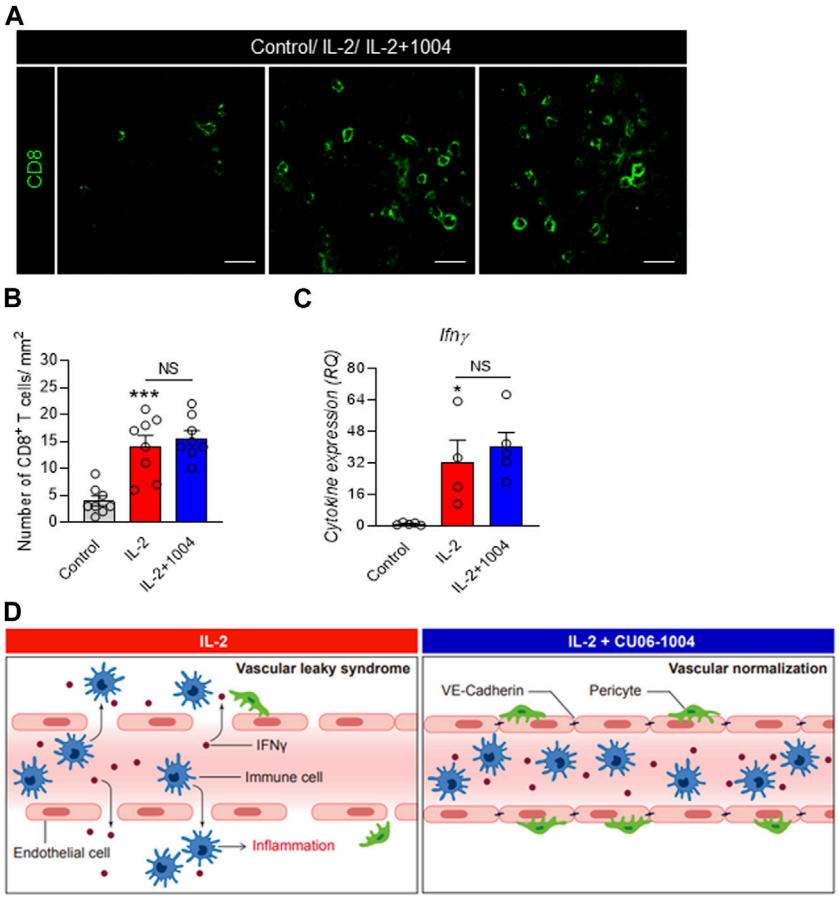

FIGURE 5
IL-2 and CU06-1004 combination therapy changes T cell infiltration and pro-inflammatory cytokine level. **(A)** Immunofluorescence staining data showed the accumulation of $CD8^+$ T cells in the tumor site in the combination treatment group. Immunofluorescence was imaged via confocal microscopy (Carl Zeiss 880, Germany). **(B)** Representative graph showing the percentages of $CD8^+$ T cells. $n = 8$ per group. Statistical analysis by one-way ANOVA with Tukey's multiple comparisons. **(C)** ELISA demonstrated a significant difference in the serum levels of IFNγ. n = 4-5 per group. Statistical analysis by one-way ANOVA with Tukey's multiple comparisons. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. ns, not significant. Data are presented as ± SEM. **(D)** In the absence of CU06-1004, IL-2 induced endothelial cell damage and vascular permeability. However, injection of a combination of IL-2 and CU06-1004 maintained the endothelial cell viability and decreased the vascular permeability.

that the administration of CU06-1004 together with IL-2 injection reduced the formation of actin stress fibers and inhibited vascular leakage through F-actin and VE-cadherin staining. In summary, the abnormal permeability caused by IL-2-induced endothelial cell damage *in vitro* was restored by CU06-1004 treatment.

Although the anticancer effect of IL-2 immunotherapy has been reported to be excellent for a long time, continuous administration is impossible because of serious side effects such as VLS (Pires et al., 2021). Therefore, we constructed an IL-2-induced VLS mouse model to enable long-term administration of IL-2 immunotherapy by reducing the side effects of IL-2 in patients. To confirm the permeability of the IL-2-induced VLS model, we injected Evans blue. To confirm permeability in the IL-2-induced VLS model, Evans blue was injected into the tail vein. Our results showed that blue dye leakage from the tissue and skin induced by IL-2 was limited by the vascular stabilization induced by CU06-1004. Thus, combination therapy with CU06-1004 in a mouse model suggests the possibility of long-term administration of IL-2 immunotherapy by alleviation of VLS.

However, although the VLS-reducing effect of CU06-1004 in normal mice is interesting, it is essential to observe its impact in a tumor-bearing mouse model for potential clinical use. Therefore, we proceeded with the co-administration of IL-2 and CU06-1004 from day 7 after injecting the B16F10 melanoma tumor into the mice. Interestingly, the Evans blue staining results revealed a reduction in vascular leakage in tumor-bearing mice treated with the combination of IL-2 and CU06-1004. This result suggests the possibility of improving the side effects of IL-2 drugs in cancer patients.

Additionally, we aimed to analyze whether the CU06-1004 and IL-2 combination group could maintain or enhance the anti-cancer effect of IL-2 while inhibiting VLS. To do so, we investigated tumor growth and size between the groups in a tumor-bearing mouse model. Surprisingly, the combination treatment with CU06-1004 demonstrated the same extent of tumor size suppression as the treatment with IL-2 alone, and in some cases, it even enhanced the suppression. Consequently, we compared the number of immune cells expected to influence tumor growth and size changes. We found that changes in tumor size correlated with the

number of cytotoxic $CD8^+$ T cells in both the IL-2-alone and CU06-1004-combined treatment groups.

Next, we analyzed the expression of pro-inflammatory cytokines that are expected to be influenced by cytotoxic $CD8^+$ T cells, using ELISA. As anticipated, the injection of IL-2 significantly increased the expression of IFNγ in mouse serum, indicating an immune activation response. Importantly, these results were also observed in the group administered the combination therapy with CU06-1004, suggesting that co-administration does not compromise the immunomodulatory effects of IL-2.

In conclusion, the co-administration of CU06-1004 and IL-2 shows promise for cancer treatment. It not only mitigates vascular leakage, which is a crucial concern with IL-2 treatment but also maintains or enhances the anti-cancer efficacy of IL-2. These findings highlight the potential of combination therapy with CU06-1004 to provide a more effective and long-term treatment option for cancer patients.

5 Conclusion

We demonstrate the suppression of high-dose IL-2-induced side effects and the improvement of anti-cancer effects through combined CU06-1004 and IL-2. In other words, a combination of CU06-1004 and IL-2 drugs is a new promising strategy to reduce severe VLS and maintained the immune response to cancer for a long time.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by Institutional Animal Care and Use Committee at Yonsei University (Permit number: IACUC-A-202104-1252-01). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

Y-GK provided expertise and research financing and contributed to writing the manuscript. SP performed all experiments and quantifications and wrote the manuscript. SL contributed to the experiment, quantification. DK and HK helped with the experiment. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Author HK and Y-GK were employed by the company Curacle Co., Ltd.

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

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Study of prevalence and risk factors of chemotherapy-induced mucositis in gastrointestinal cancer using machine learning models

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Objective: Chemotherapy-induced mucositis (CIM) significantly impacts clinical outcomes and diminishes the quality of life in patients with gastrointestinal cancer. This study aims to prospectively determine the incidence, severity, and underlying risk factors associated with CIM in this patient population.

Methods: To achieve this objective, we introduce a novel Machine Learning-based Toxicity Prediction Model (ML-TPM) designed to analyze the risk factors contributing to CIM development in gastrointestinal cancer patients. Within the winter season spanning from December 15th, 2018 to January 14th, 2019, we conducted in-person interviews with patients undergoing chemotherapy for gastrointestinal cancer. These interviews encompassed comprehensive questionnaires pertaining to patient demographics, CIM incidence, severity, and any supplementary prophylactic measures employed.

Results: The study encompassed a cohort of 447 participating patients who provided complete questionnaire responses (100%). Of these, 328 patients (73.4%) reported experiencing CIM during the course of their treatment. Notably, CIM-induced complications led to treatment discontinuation in 14 patients (3%). The most frequently encountered CIM symptoms were diarrhea (41.6%), followed by nausea (37.8%), vomiting (25.1%), abdominal pain (21%), gastritis (10.5%), and oral pain (10.3%). Supplementary prophylaxis was administered to approximately 62% of the patients. The analysis revealed significant correlations between the overall incidence of CIM and gender (p=0.015), number of chemotherapy cycles exceeding one (p=0.039), utilization of platinum-based regimens (p=0.039), and administration of irinotecan (p=0.003). Specifically, the incidence of diarrhea exhibited positive correlations with prior surgical history (p=0.037), irinotecan treatment (p=0.021), and probiotics usage (p=0.035). Conversely, diarrhea incidence demonstrated an adverse correlation with platinum-based treatment (p=0.026).

Conclusion: In conclusion, this study demonstrates the successful implementation of the ML-TPM model for automating toxicity prediction with

accuracy comparable to conventional physical analyses. Our findings provide valuable insights into the identification of CIM risk factors among gastrointestinal cancer patients undergoing chemotherapy. Furthermore, the results underscore the potential of machine learning in enhancing our understanding of chemotherapy-induced mucositis and advancing personalized patient care strategies.

KEYWORDS

chemotherapy side effects, chemotherapy toxicity, cancer treatment toxicity, chemotherapy tolerability, gastrointestinal cancer, chemotherapy-induced mucositis, incidence, risk factors

Introduction

Gastrointestinal (GI) cancer stands as a significant contributor to cancer-related morbidity and mortality on a global scale. Conventional chemotherapy has been recommended for patients exhibiting poor prognostic indicators, aiming to mitigate the risk of tumor recurrence and progression (1). Among the challenges posed by such therapies, mucositis emerges as a prevalent concern, characterized by ulceration and erythema of the mucosal lining within the GI tract. This affliction manifests in approximately 20-40% of patients undergoing traditional chemotherapy regimens (2, 3). Distinctively, oral mucositis is typified by ulceration and erythema affecting the oral mucosa, while GI mucositis commonly presents with symptoms encompassing pain, vomiting, nausea, and diarrhea. Given the widespread usage of cytotoxic treatments in GI cancer cases, an increased in the trajectory in the occurrence of chemotherapy-associated mucositis (CIM) has given rise to a pressing clinical issue (4). This issue brings significance changes specially detrimental impact on patients' quality of life, treatment adherence, prolonged hospital stays, and overall clinical outcomes (5, 6).

Certain cytotoxic drugs such as platinum, irinotecan, and fluorouracil frequently cause CIM when FOLFOX, FOLFIRI, and S-1 are recommended during proper management of GI malignancies. Due to tumor occupation or surgical damage, CIM in GI cancer patients would aggravate the damage of the patient's GI function and impaired prognosis compared with non-digestive tumors such as head and neck, and lung cancer. In previous studies that were focused on wild-type tumors, higher incidence rates of oral mucositis and diarrhea were observed (7–9). Some of the previously published studies focused on individual components of CIM revealed that the incidence and severity rate varied from patient to patient and those were regime-related. Potential risk factors observed such as gender, age, past medical history, as well as the type of drug used, its dosage, schedule, and administration route (10–12). However, even Multinational Association Of Supportive Care In Cancer and International Society of Oral Oncology (MASCC/ISOO) (2) and European Society for Medical Oncology (ESMO) clinical practice guidelines (13) for the management of oral and GI mucosa injury have proposed limited measurements mainly on the head and neck radiation-related and hematopoietic stem cell transplantation related mucositis.

Until now, the treatment for CIM consists of diverse drugs, not only having no established standard schedule but presenting inconsistent outcomes. Glutamine, probiotics, and a comprehensive elemental diet are widely applied as countermeasures for CIM (14). However, even those prescribed drugs should be pre-arranged before chemotherapy. Moreover, these drugs are usually administered unless severe complications occur and may themselves transiently bring about adverse effects, including pruritus, rash, erythema, tongue and mouth disorders, and taste alteration (15).

In recent years, machine learning (ML) and artificial intelligence (AI) have been used to forecast chemotherapy-related complications (16, 17). Owing to the early adoption of electronic chemotherapy recommendations, a rich source of past patient information regarding chemotherapy and gastrointestinal cancer and a subset of key parameters has been established. Novel data mining techniques incorporating ML methods can be utilized to analyze these data to produce more accurate, personalized predictions of the risk of gastrointestinal cancer. Machine learning can forecast the recurrence of gastric cancer patients after an operation.

There are very few studies focusing on the occurrence of CIM in GI cancers. Therefore, the current cross-sectional study is designed to obtain the overall incidence and severity of CIM in patients with gastrointestinal cancer. In addition, clinical features covering patient characteristics, inducements, and therapeutic factors of high-risk populations have also been examined. Hence, in this study, ML-based Toxicity Prediction Model (ML-TPM) has been proposed for analyzing the risk factors of CIM in GI cancer.

Materials and methods

Patients

This study enrolled consecutive in-hospital patients with gastric or colorectal cancer who had received at least one cycle of chemotherapy in the cancer center in our Hospital from Dec. 2018 to Jan. 2019. The Institutional Ethics Committee of the Hospital approved the protocol. The prior consent of the

participants was taken before participation in the study. The exclusion criteria implied was: Patients with an ECOG score >2 had vomiting, diarrhea, or gastrointestinal bleeding before chemotherapy, with uncontrolled thyroid, diabetes, kidney or liver disorder. All included patients were interviewed via questionnaires which were face-to-face and recorded by two well-trained study nurses about (1) Personal information including age, gender, height, weight, and ECOG score; (2) Previous disease history including surgery or radiation history, other medical and medication history; (3) Disease info included tumor location, Tumor, Node, Metastasis (TNM) stage, chemotherapeutic regimens, and cycles were traced through electronic medical records system; (4) Symptomatic info covered the onset, duration, extent, and management of CIM like nausea and vomiting, diarrhea, abdominal pain, oral pain, and gastritis.

Chemotherapeutic regimens and CIM management

In this study, we selected chemotherapeutic regimens by treating physicians according to clinical practice guidelines FOLFIRI regimen (irinotecan 150 mg/m^2 i.v. on day 1, leucovorin at a dose of 200 mg/m^2 i.v. on day 1, followed by bolus 5-FU400 mg/m^2, and a 46 h infusion of 5-FU (2400 mg/m^2) on days 1 to 2 were administered every 2 weeks) or mFOLFOX6 regimen (oxaliplatin 85 mg/m^2 i.v. on day 1, leucovorin 100 mg/m^2 i.v. on days 1 & 2 trailed by bolus 5-FU 400 mg/m^2, and a 46-h infusion of 5-FU 2400 mg/m^2 on days 1 to 2 were administered every 2 weeks) and XELOX regimen (Xeloda 2000 mg/m^2/d for 1-14 days. A 2 h infusion of oxaliplatin (130 mg/m^2) i.v. Day 1 for every 3 weeks was utilized for patients with colorectal cancer. SOX regimen (S1 80 mg/m^2 on days 1 to 14 po. bid. and a 2 h infusion of oxaliplatin (130 mg/m^2) i.v. on day 1 every 3 weeks) or XELOX regimen was used for gastric cancer. CIM management, including glutamine, probiotics, enteral nutrition, digestive enzymes, and Chinese herbs, was took by the patients.

Endpoints and statistical analysis

In the current study, the first endpoint was the incidence of CIM at any grade of targeted CIM symptoms, including oral pain, abdominal pain, gastritis, nausea, vomiting, and diarrhea. Grades of CIM were scaled consistently with the National Cancer Institute Common Terminology Criteria for Adverse Event v4.0 (NCI-CTCAE v4.0). Secondary endpoints include correlation factors for CIM.

Both primary and secondary endpoints were analyzed as described previously (18). The accuracy of the TPM model was in ML-TPM setting was assessed as described previously (19).

Statistical analysis

Data were set as ordered variables and were entered into a computerized database (SPSS statistical software, SPSS Inc., Chicago, IL). Kendall test was applied to detect the correlation between the incidence of CIM and patient demographics, including baseline information, chemo-regimens, and supplementary CIM prophylaxis. Statistical implication was accepted at the p <0.05 levels.

Results

Patients

A total of 447 patients with gastric (31.5%) or colorectal cancer (68.5%) who were scheduled for chemotherapy were timely interviewed between Dec. 2018 and Jan. 2019, and 100% valid questionnaires were collected and valid. The baseline characteristics have also been tabulated (Table 1). The middle age observed was 56 yrs. The mainstream of patients had normal nutritional status with a normal BMI score (74.9%) and lower ECOG score (ECOG=0, 85.7%; ECOG=1,13.6%), and the number of patients with stage IV disease was relatively high (48.3%). In previous treatment history, 329 of 447 patients (73.6%) had surgery, 61 patients (13.6%) received target therapy, and 89 patients (19.9%) had a history of radiotherapy. Large majority of the patients (62%, n=277), out of 447 patients received supplementary drugs (Table 2).

TABLE 1 Baseline characteristics.

Variable	N,(%)
Age,median,years	56
<70	399(89.3)
≥70	48(10.7)
Gender	
Male	278(63.2)
Female	169(37.8)
BMI[a]	
<18	37(8.3)
18~22.9	335(74.9)
23~24.9	66(14.8)
≥25	9(2)
ECOG[b]	
0	383(85.7)
1	61(13.6)
2	3(0.7)
Diagnosis	
Gastric cancer	141(31.5)
Colorectal cancer	306(68.5)
Tumor stage	
Stage I	7(1.6)
Stage II	47(10.5)

(Continued)

TABLE 1 Continued

Variable	N,(%)
Stage III	160(35.8)
Stage IV	216(48.3)
Unknown	17(3.8)
Chemotherapy regimens	
Platinum Based	358(80.1)
Irinotecan Based	63(14.1)
Fu-i.v. Based	156(34.9)
Fu-oral Based	274(61.3)
Cycles	
1 cycle	91(20.4)
>1 cycle	356(79.6)
Previous treatment	
Operation	329(73.6)
Target agent	
Bevacizumab	47(10.5)
Cetuximab	12(2.7)
Radiotherapy	89(19.9)
Chinese herbs	147(32.9)

[a]BMI, Body Mass Index.
[b]ECOG, Eastern Cooperative Oncology Group.

Incidence rate and severity of CIM

In this study, 119 patients (26.6%) did not display any CIM symptoms during the chemotherapy courses. 328 patients (73.4%) suffered from CIM (Table 3), and 14 patients (3.1%) had to discontinue treatment as a result of intolerable CIM. The highest overall incidence of CIM was diarrhea (41.6%), nausea (37.8%), vomiting (25.1%), abdominal pain (21%), gastritis (10.5%), and oral pain (10.3%). Severe CIM (grade ≥3) happened in 4 patients (0.9%) with oral pain, 3 patients (0.6%) with abdominal pain (2 with grade 4 malaise), 2 patients (0.4%) with nausea, 3 patients (0.7%) with vomiting, 16 patients (3.6%) with diarrhea (3 with grade 4 malaise), no patient presented severe gastritis.

TABLE 2 Proportion of supplementary prophylaxis.

Supplementary prophylaxis	N(%)
Enteral nutrition	157(35.1)
Probiotics	99(22.1)
Glutamine	21(4.7)
Digestive enzyme	34(7.6)
Vitamines	62(13.9)
Chinese herbs	147(32.9)

The correlation between CIM incidence and patients' clinical characteristics

Being a highly concerning issue, the overall incidence of CIM was significantly correlated with gender (p=0.015), chemotherapy cycles (p=0.039), platinum-based (p=0.039), and irinotecan-based treatment (p=0.003). No correlation was detected between the overall incidence of CIM and the administration of oral fluorouracil. We further analyzed the association of clinical characteristics and incidence of each type of CIM. Firstly, we investigated the correlation between all kinds of CIM and patients' baseline characters (Table 4). The incidence of oral pain was positively correlated with poor ECOG and chemotherapy cycles. The incidence of abdominal pain was positively interrelated with gender/female and higher TNM stage. In the incidence of gastritis, a significant positive correlation was revealed between higher ECOG scores and chemotherapy cycles. A negative correlation was revealed with tumor location/colorectal location and a history of surgery. The incidence of nausea and vomiting presented some statistical similarities; they positively correlated with female gender and chemotherapy cycles >1 and negatively with older age. The surgical history was revealed as a risk factor for diarrhea. Among all the baseline characters, chemotherapy cycles>1 and gender/female were detected as significantly correlated with the incidence of varied CIM types, indicating that patients with multiple lines of chemotherapy and female patients should draw more attention to the high risk of CIM.

Next, the incidence of subtypes of CIM varies with different chemotherapy regimens (Table 5). Platinum-based chemotherapy was positively linked with the incidence of abdominal pain and negatively linked with the incidence of diarrhea. Irinotecan-based chemotherapy was positively linked with the incidence of overall CIM and diarrhea, while negatively interrelated with the incidence of oral pain and abdominal pain; Fu-i.v. based chemotherapy was only positively correlated with oral pain; Fu-oral based treatment was negatively correlated with the incidence of oral pain and gastritis. The predictive model used, along with its accuracy, was depicted in Figures 1, 2.

The correlation between CIM incidence and supplementary prophylaxis

In the current study, 277 (62%), out of 447 patients received supplementary drugs, including enteral nutrition, glutamine, probiotics, digestive enzymes, and Chinese herbs. Among them, the patients who choose enteral nutrition are the most, accounting for 35.1%. The impact of supplementary prophylaxis on the incidence of CIM was also recorded (Table 5). There was no statistically important correlation between the total incidence of CIM and the administration of supplemental elements. Current results were unexpected and demonstrated that oral pain was positively associated with a digestive enzyme and vitamin intake, and diarrhea incidence was positively associated with probiotics intake.

TABLE 3 The incidence of chemotherapy-related mucositis.

	Incidence of CIM,n (%)				
	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Oral pain	401(89.7)	37(8.3)	5(1.1)	4(0.9)	/
Abdominal pain	353(79)	73(16.3)	18(4)	1(0.2)	2(0.4)
Gastritis	400(89.5)	43(9.6)	4(0.9)	0	0
Nausea	278(62.2)	143(32)	24(5.4)	2(0.4)	/
Vomiting	335(74.9)	92(20.6)	17(3.8)	3(0.7)	0
Diarrhea	261(58.4)	141(31.5)	29(6.5)	13(2.9)	3(0.7)
	None	Any symptom of CIM			
Total	119(26.6)	328(73.4)			

Discussion

The present study reported a high incidence of CIM (73.4%) in gastric or colorectal cancer in Chinese patients receiving combination chemotherapy. However, severe CIM was rare (0.4-3.6%), and only 14 patients (3.1%) discontinued chemotherapy due to intolerable CIM; this fact mimics previous findings (11). Despite that low-grade CIM was dominant, they present as oral or gastrointestinal adverse effects that could impair patients' quality of lives, dosage tolerance, and motivation for treatment and may ultimately result in worse survival.

Herein, it was found that clinical characteristics for CIM to identify high-risk GI cancer patients for preventing management. The overall incidence of CIM significantly positively correlated with gender and chemotherapy cycles; female and chemotherapy cycle>1 were highlighted as risk factors for CIM and diverse CIM types, and the findings remained consistent with the literature (20). Up to now, certain systematic reports on the vulnerability factors of CIM were published, most of which just focused on partial symptoms of it, such as oral mucositis (OM) and diarrhea, and tend to be reported unless severe conditions occurred. Few studies suggested that advanced age, a lack of craving, and the duration of chemotherapy might contribute to OM in breast, lung, and gastrointestinal tract cancer. At the same time, tumor-specific correlations are not mentioned (21). The history of chemotherapy and the number of chemotherapy cycles was regarded as involved in developing CIM (21, 22). Among the GI cancer population, we found patients who expected more than 1 cycle of chemotherapeutic displayed a high incidence of overall CIM and oral pain, gastritis, nausea, and vomiting. Meanwhile, female patients presented a high risk of overall CIM, abdominal pain, nausea, and vomiting. Besides, younger patients showed a higher incidence of nausea and vomiting. Considering the similar trend in women, we suggest defining young females as a high-risk population for CIM. Tumor location, TNM stage, and surgery history had no significant correlation with the overall incidence of CIM but correlated with some malaise like gastritis and abdominal pain, respectively, which were recognized free of chemotherapy inducement but more relevant to disease or operation factors. Notably, a history of target agents and radiotherapy did not increase CIM incidence in our population, inconsistent with some articles which believe target and radiation therapy can lead to mucositis (13).

Based on our analysis, platinum and irinotecan significantly correlated with CIM incidence in opposite directions. Platinum-based treatments were negatively correlated with an overall incidence of CIM and diarrhea, while irinotecan-based treatment positively correlated with an overall incidence of CIM and diarrhea. 5-FU-based treatment was not correlated with the incidence of CIM, whether administered intravenously or orally. Among the chemotherapy agents recommended utilized for patients with GI cancer, oxaliplatin, irinotecan, and 5-FU are three drugs with the maximum risk for CIM, with their direct or indirect damage leading to the breakdown of the mucosal barrier, crypt cell death, and lastly, mucosal inflammations (11). Chemotherapy-induced diarrhea and OM are usually reported with 5-fu and irinotecan (8, 23), and nausea and vomiting are published more with irinotecan and oxaliplatin (24). The present study showed irinotecan had been related to multiple CIM sub-symptoms: diarrhea (positively), oral pain, and abdominal pain (negatively). Regarding the development of oral pain, 5-FU given through oral acted negatively rather than intravenous delivery. Thus, we would like to conclude that it's necessary to pay more attention to CIM toxicities in the course of irinotecan involving chemotherapy and intravenous administrated of 5-FU.

In this cross-sectional study, an initial observation was that a high proportion (62%) of supplements includes glutamine, probiotics, enteral nutrition, digestive enzymes, and Chinese herbs in GI patients to alleviate or prevent CIM. However, we did not obtain a beneficial correlation between all mentioned supplementary drugs and the incidence of CIM or CIM sub-symptom. Due to insufficient evidence, management standards regarding CIM in gastrointestinal cancer are still inaccessible and inconsistent. A mass of articles discussed complementary agents for treating CIM. Glutamine could protect bowel mucosa from chemotherapy-induced DNA damage through the production of reactive oxygen species and apoptosis through the expression of inflammatory cytokines, such as tumor necrosis factor α (TNFα) and interleukin (IL)-1β, IL-6 (25, 26). Probiotics also counter the

TABLE 4 The relevance of baseline characteristics and chemotherapy-related mucositis.

	Overall		Oral pain		Abdominal pain		Gastritis		Nausea		Vomiting		Diarrhea	
	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value	Correlation coefficient	P-value
Age	-0.07	0.103	0.046	0.232	-0.058	0.135	0.037	0.341	-0.13	0.001	-0.114	0.003	-0.031	0.453
Gender	0.115	0.015	0.041	0.384	0.107	0.021	0.041	0.384	0.118	0.011	0.137	0.003	0.007	0.886
BMI	-0.037	0.421	-0.01	0.798	-0.054	0.158	<0.0001	0.994	-0.041	0.282	-0.071	0.062	-0.044	0.323
ECOG	0.043	0.362	0.116	0.013	0.069	0.138	0.111	0.018	0.017	0.709	-0.066	0.157	0.071	0.116
Tumor location	-0.08	0.094	0.072	0.124	<0.0001	0.992	-0.126	0.007	0.077	0.095	-0.007	0.883	-0.05	0.271
TNM stage	0.078	0.089	0.056	0.227	0.122	0.008	0.015	0.748	0.053	0.253	-0.007	0.883	-0.036	0.408
Chemotherapy cycle	0.098	0.039	-0.151	0.001	0.05	0.28	0.101	0.032	0.128	0.006	0.112	0.016	-0.062	0.176
Surgery	0.024	0.561	-0.043	0.286	-0.077	0.06	-0.084	0.044	0.031	0.448	0.002	0.961	0.084	0.037
Target agents	-0.019	0.686	-0.016	0.728	-0.064	0.172	-0.031	0.51	-0.052	0.268	-0.056	0.228	0.003	0.953
Radiotherapy	-0.004	0.935	-0.025	0.6	-0.037	0.43	-0.043	0.363	0.085	0.073	0.074	0.12	0.036	0.341

TABLE 5 The relevance of chemotherapy regimen and supplementary prophylaxis with chemotherapy-related mucositis.

Parameters	Overall		Oral pain		Abdominal pain		Gastritis		Nausea		Vomiting		Diarrhea	
	Correlation coefficient	*p*-value	Correlation coefficient	*p*-value	Correlation coefficient	*p*-value	Correlation coefficient	*p*-value	Correlation coefficient	*p*-value	Correlation coefficient	*p*-value	Correlation coefficient	*p*-value
Platinum Based	-0.098	0.039	0.051	0.275	0.113	0.015	0.086	0.068	0.021	0.643	0.002	0.966	-0.101	0.026
Irinotecan Based	0.142	0.003	-0.1	0.034	-0.143	0.002	-0.032	0.5	-0.033	0.48	-0.059	0.203	0.105	0.021
Fu-i.v. Based	0.016	0.731	0.154	0.001	0.019	0.682	-0.02	0.668	0.01	0.826	-0.024	0.605	-0.039	0.394
Fu-oral based	-0.032	0.502	-0.167	<0.0001	-0.044	0.34	-0.042	<0.0001	0.009	0.843	0.018	0.695	0.045	0.325
Enteral nutrition	0.019	0.687	-0.038	0.421	0.046	0.333	0.023	0.63	-0.023	0.63	-0.047	0.322	-0.051	0.285
Probiotics	0.06	0.202	0.054	0.256	-0.007	0.886	0.054	0.256	0.031	0.519	0.031	0.518	0.1	0.035
Glutamine	-0.01	0.836	-0.007	0.88	-0.011	0.82	-0.007	0.88	0.001	0.978	0.018	0.704	-0.016	0.738
Digestive enzyme	0.077	0.102	0.259	<0.0001	0.1	0.034	0.067	0.159	0.02	0.674	-0.03	0.532	0.083	0.079

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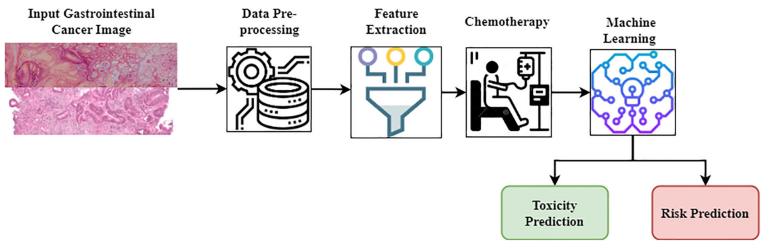

FIGURE 1
Proposed ML-TPM model. In the current study, the Kaggle dataset of gastrointestinal cancer images used to predict the prevalence and risk factors in clinical outcomes. Before deploying the ML model, it is essential to ensure the quality of the datasets using a preprocessing technique that includes feature extraction and standardization. Without any self-learning system, the feature extraction of a GI endoscopic image relies heavily on color and texture information. Chemotherapy (chemo) is the treatment of cancer using anti-cancer medications, either intravenously (through an IV line or central venous catheter) or orally (in the form of tablets). It is possible to treat cancer that has spread to other organs by using medications circulating throughout the body through circulation. Researchers are increasingly turning to machine learning to predict toxicity in gastrointestinal cancer due to the method's speed, low cost, and high accuracy. A ML model is first developed to forecast toxicity after the chemical structure is represented using a computer-readable and interpretable technique. The primary treatment for gastrointestinal cancer is often systemic anti-cancer medications, with surgery, neo-adjuvant (chemotherapy), and postoperative adjuvants (chemotherapeutic) for high-risk improved stages (high-risk stage III and II).

pathophysiology of mucositis with the effect of relieving dysbacteriosis caused by chemo-treatment. A recent meta-analysis study (27) has revealed that probiotics decreased the occurrence of diarrhea in cancer patients (95% CI 0.34-0.78 OR=0.52). Chinese herbs such as Rhodiolaalgida may possess anti-inflammation effects and quickly heal mucosa ulcers (28, 29). However, none of them raise high-quality evidence for their limitations in sample size or inadequate design. On the contrary, results in the present research failed to verify the relationship between supplementary prophylaxis and overall CIM incidence. At the same time, probiotics, digestive enzymes, and vitamins were significantly correlated with sub-types of CIM, despite non-beneficial effects. Considering varied types of supplementary drugs having many compounds, dosages, and pharmacodynamics, it is pretty hard to conduct further stratified analyses to discover potentially effective drugs. To determine the effect of supplementary prophylaxis on CIM, a prospective, multiple-center, randomized, controlled clinical trial for more valid evidence for preventing the management of CIM was crucial.

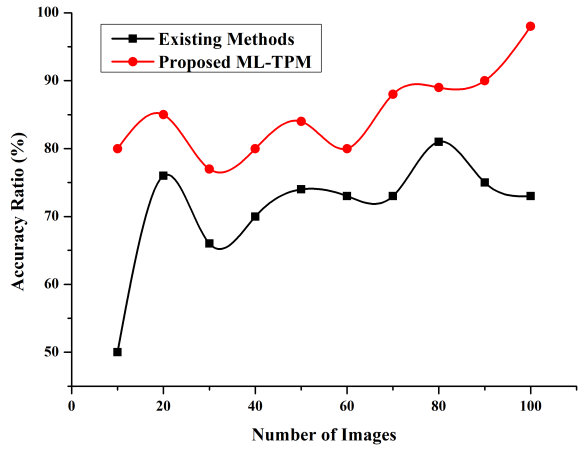

FIGURE 2
Accuracy Ratio. Developing a ML method that can automatically recognize lesion images from substantial GI cancer image datasets is necessary and meaningful to enhance detection efficiency and accuracy. The y-axis represent % accuracy ratio, and the x-axis represents the number of the images. The red colored line denotes proposed ML-TPM, and black line indicates existing methods.

Conclusion

This study was focused on application of the Machine Learning-based Toxicity Prediction Model (ML-TPM), designed to analyze the underlying risk factors associated with chemotherapy-induced mucositis (CIM) in GI cancer patients. Our cross-sectional assessment highlights a prevalent occurrence of CIM among patients undergoing chemotherapy for gastrointestinal cancer. Notably, female patients, those subjected to more than one chemotherapy cycle, and those treated with irinotecan or platinum-based regimens exhibit heightened susceptibility to CIM. Furthermore, we observe that a substantial portion (50%) of patients opt for supplementary prophylactic measures to manage CIM symptoms. It remains imperative to investigate the potential efficacy of supplementary prophylaxis, particularly for high-risk patients. Nevertheless, it's important to acknowledge the limitations of this observational study, including its modest sample size, short-duration timeframe, and single-institute design. Additionally, variability and irregularity in the use of supplementary drugs for CIM by patients add complexity to the findings. To enhance the rigor of our findings, we recommend the execution of a prospective interventional study involving a larger and more diverse patient population. The noteworthy positive correlations established in this study were between CIM incidence, gender, and chemotherapy cycles in concordance with the previous study. In particular, our study underscores female gender and undergoing more than one chemotherapy cycle as significant risk factors for diverse CIM types. Looking ahead, our research paves the way for future investigations into biomarkers that could facilitate the optimization of precision treatment strategies for gastrointestinal cancer through computer-aided diagnostic tools. This pursuit holds the potential to contribute

significantly to advancing personalized therapeutic approaches in this domain.

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 humans were approved by Ethics Committee of West China Hospital of Sichuan University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

Study conception and design: LH, XY. Data collection: FW, XW. Analysis and interpretation of results: LH, XY, MQ. Draft manuscript preparation: MQ. All authors reviewed the results and approved the final version of the manuscript.

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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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Case Report: Resolution of remitting seronegative symmetrical synovitis with pitting edema during nivolumab therapy for gastric cancer

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The anti-programmed cell death-1 (PD-1) antibody nivolumab has been shown to significantly prolong the survival of patients with unresectable advanced or recurrent gastric cancer (AGC). However, immune-related adverse events (irAEs), which show different profiles from those of cytotoxic agents or conventional molecular-targeted drugs including tyrosine kinase inhibitors, have been reported. Remitting seronegative symmetrical synovitis with pitting edema (RS3PE) is a rare autoimmune disorder with acute-onset, rheumatoid factor-negative, symmetric synovitis associated with limb edema observed in elderly persons. A case of RS3PE syndrome that developed after administration of nivolumab for advanced gastric cancer is reported. This is the first report of a case of RS3PE syndrome as an irAE caused by nivolumab in a patient with gastric cancer.

KEYWORDS

gastric cancer, RS3PE, irAE, nivolumab, pathology

1 Introduction

For patients with advanced or recurrent gastric cancer, systemic chemotherapy is the standard therapy. Platinum, fluoropyrimidine, and taxanes are used as cytotoxic agents. Ramucirumab, an angiogenesis inhibitor, is used for human epidermal growth factor receptor 2(HER2)-positive or negative advanced or recurrent gastric cancer (AGC), and trastuzumab and trastuzumab deruxtecan are used for HER2-positive AGC as molecular-targeted agents (1–5). In recent years, the efficacy of immune checkpoint inhibitors against

AGC, which is resistant to these agents, has been shown. Nivolumab, a fully human anti-programmed death-1 (PD-1) monoclonal antibody, demonstrated a significant survival benefit for previously untreated AGC patients in combination with chemotherapy and for AGC patients previously treated with two or more chemotherapy regimens (6–8). It is considered that the administration of anti-PD-1 antibody activates tumor-specific T cells in cancer-bearing patients, resulting in an antitumor effect. On the other hand, it is also known that immune-related adverse events (irAEs) based on the autoimmune response, including skin disorders, colitis, and thyroid dysfunction, develop due to non-tumor-specific T cell activation (9), and attention to irAEs is needed.

Remitting seronegative symmetrical synovitis with pitting edema (RS3PE), a disease reported by McCarty et al., occurs more frequently to men over the age of 60 years (male to female ratio, 3:2), and patients develop acute or subacute synovitis accompanied by symmetric pitting edema of the limbs or feet. RS3PE is negative for serum rheumatoid factor and joint destruction on X-ray images and has a good prognosis. RS3PE has a similar pathology to polymyalgia rheumatica (PMR), except that the predominant manifestation of PMR is arthritis. In the treatment of RS3PE, administration of steroids is effective. It has also been reported that 16-30% of patients with RS3PE are associated with malignant tumors such as prostate cancer, colon cancer, gastric cancer, and hematopoietic tumors (10). RS3PE patients with malignant tumors are often resistant to treatment, but some cases showed improvement of symptoms after tumor resection (11). Based on these characteristics, RS3PE is considered one of the paraneoplastic syndromes. However, the mechanism of its onset has still not been elucidated.

RS3PE that developed after administration of nivolumab has been reported in three cases of malignant melanoma and one case of non-small cell lung cancer (12–15). However, there has been no report of a gastric cancer case that developed RS3PE after the administration of nivolumab. In addition, histological evaluation of an RS3PE case after administration of nivolumab has not been performed. The first gastric cancer case that developed RS3PE after the administration of nivolumab is presented.

2 Case description

A 73-year-old man developed black stool in January 2017 and consulted a family doctor. Upper gastrointestinal endoscopy showed type 3 advanced gastric cancer (pathological diagnosis: moderately to poorly differentiated adenocarcinoma, HER2-negative) with main lesions from the body to the antrum of the stomach. He had hypertension, aortic stenosis, type II diabetes mellitus, and dyslipidemia since the age of about 50 and he was treated by a family doctor. There was no family history of malignant tumor or collagen disease. He smoked about one pack of cigarettes a day for 48 years. He was referred to our hospital for treatment. His general condition was Eastern Cooperative Oncology Group performance status (ECOG PS) 1, and there were no abnormal physical findings other than pallor of the palpebral conjunctiva.

On close examination, the primary lesion and regional lymph node metastasis were found, and the clinical stage was diagnosed as cT4N3aM0, cStage IIIB. Though laparoscopic curative surgery was planned, peritoneal dissemination was observed during the operation, and he was diagnosed with unresectable gastric cancer (sT4a (SE) N2P1CY0H0, sStage IV). From February 2017, S-1 + oxaliplatin therapy (S-1: 120 mg/day, day 1-14, oxaliplatin: 100 mg/m^2, day 1, 3-week cycle) was administered.

In April, a tonic-clonic seizure occurred after the second administration of S-1+oxaliplatin. No abnormalities were found on head magnetic resonance imaging, but it could not be ruled out that S-1 was the cause, and chemotherapy was switched to second-line therapy with paclitaxel. An increase in size of abdominal lymph nodes was observed on computed tomography (CT), and progressive disease (PD) was confirmed by response evaluation criteria in solid tumors (RECIST) criteria version 1.1 (16).

From November, nivolumab monotherapy (3 mg/kg, 2-week cycle) was administered as third-line chemotherapy. Tumor markers (CEA and CA19-9) decreased after 4 cycles of nivolumab, and CT showed shrinkage of abdominal metastatic lymph node lesions after 7 cycles. However, the bilateral lower leg edema appeared at the 4th cycle of nivolumab in December, and right shoulder pain appeared and persisted from late February 2018.

During the administration of nivolumab, edema of both fingers and the dorsa of both hands appeared in April 2018 (Figure 1), and pitting edema of both lower legs deteriorated. Erythema also appeared on the dorsal sides of the fingers. On ultrasonographic examination, fluid retention around the biceps, synovitis of the biceps, and synovitis of the joint synovium and tendon sheath synovium were observed (Figure 1). There were no abnormalities in thyroid hormone levels or the blood coagulation system. C-reactive protein was as high as 6.75 mg/dL, and rheumatoid factor was negative (8 U/mL). Serum autoantibodies including anti-nuclear antibody, anti-ds-DNA antibody, anti-SS-A antibody, anti-SS-B antibody, anti-Scl-70 antibody, anti-RNP antibody, anti-centromere antibody, anti-RNA polymerize III antibody, anti-neutrophil cytoplasmic antibody (MPO-ANCA and PR3-ANCA), anti-MDA antibody, anti-Mi-2 antibody, anti-TIF1-gamma antibody, and anti-ARS antibody were all negative. Human leukocyte antigen (HLA) typing showed HLA-A2 and HLA-CW7 serotypes. Immune cell subsets of peripheral blood mononuclear cells were analyzed using flow cytometry, as previously described (17), at three time points: prior to administration of nivolumab; after one cycle of treatment; and at the times of developing RS3PE syndrome. Activated memory, effector, and helper T cells tended to increase after administration of nivolumab. In addition, coinhibitory molecule (TIM3, TIGIT, and LAG3) and costimulatory molecule (OX40)-positive T cells also increased (Table 1). A skin biopsy from the dorsum of the hand showed lymphocytic infiltration around blood vessels in the surface layer of the skin and in the interstitium of the skin, and no malignant cells were observed. Multiplex immunostaining of skin tissue using

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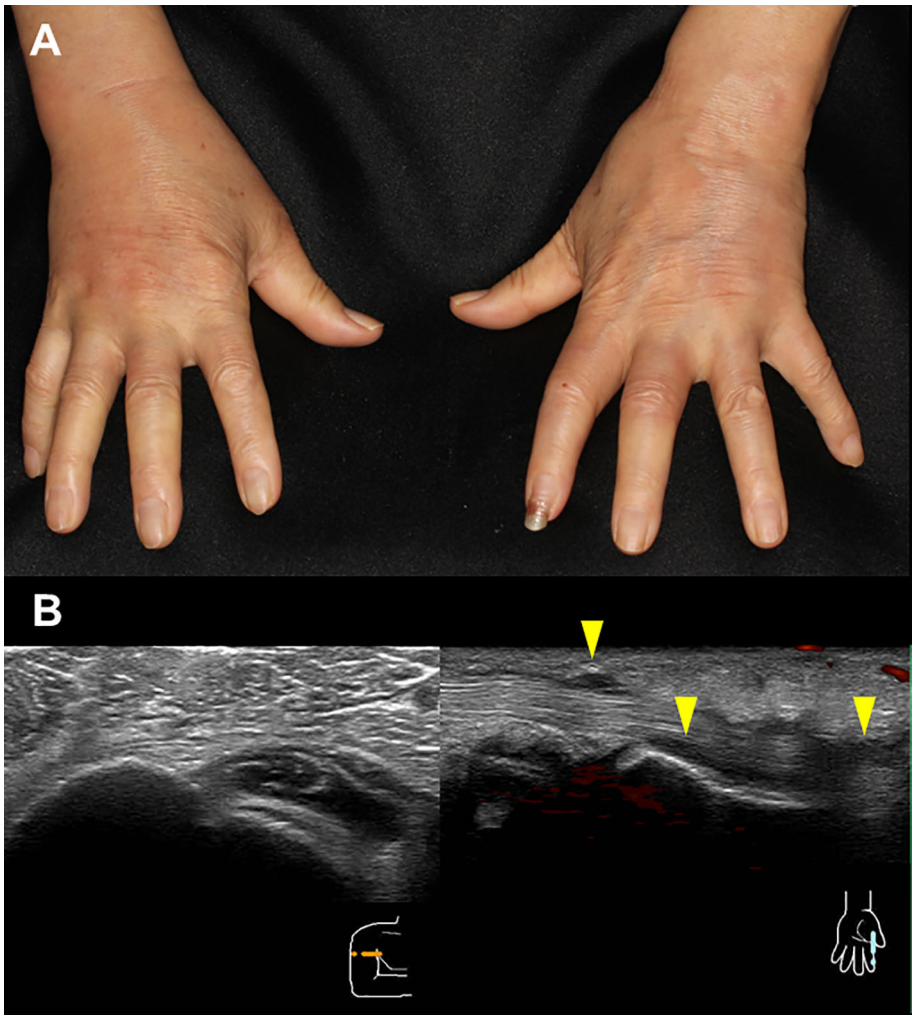

FIGURE 1
Edema of both fingers and the dorsa of both hands and erythema on the dorsal sides of the fingers **(A)**. On ultrasonographic examination, fluid retention around the biceps, synovitis of the biceps, and synovitis of the joint synovium and tendon sheath synovium are observed **(B)**.

TABLE 1 Immune cell subsets of peripheral blood mononuclear cells during therapy.

(%)	Before administration of nivolumab	After administration of nivolumab	RS3PE
T cell/PBMC	50.2	58.6	58.3
CD4+T cell/T cell	71.5	71.6	87.7
naïve CD4+T cell	26.0	28.5	42.1
activated	3.8	4.6	2.8
central memory CD4+T cell	43.0	38.1	39.9
activated	5.4	8.8	9.1
effector memory CD4+T cell	29.4	32.7	17.5
activated	18.1	22.2	35.1
effector CD4+T cell	1.8	0.9	0.5
activated	29.6	31.2	23.1

(Continued)

TABLE 1 Continued

(%)	Before administration of nivolumab	After administration of nivolumab	RS3PE
Th1/CD4+T cell	18.4	15.5	12.1
activated	10.0	11.1	4.9
Th2/CD4+T cell	58.1	62.5	70.3
activated	4.7	6.7	2.3
Th17/CD4+T cell	12.0	12.3	12.9
activated	16.3	9.8	8.9
Th1/17/CD4+T cell	11.4	9.8	4.7
activated	9.3	12.4	4.0
TIM3+CD4+T cell	17.6	15.1	25.2
LAG3+CD4+T cell	4.5	5.8	21.5
TIGIT+CD4+T cell	15.0	17.3	19.9
OX40+CD4+T cell	46.6	77.1	84.1
CD8+T cell/T cell	22.9	24.5	10.6
naïve CD8+T cell	2.8	3.7	9.9
activated	25.0	19.7	13.3
central memory CD8+T cell	24.5	17.7	23.4
activated	10.4	19.4	15.5
effector memory CD8+T cell	62.2	69.0	56.8
activated	29.5	49.9	51.7
effector CD8+T cell	11.6	9.6	9.9
activated	31.6	42.6	53.3
TIM3+CD8+T cell	15.2	17.7	31.0
LAG3+CD8+T cell	2.3	9.9	16.0
TIGIT+CD8+T cell	54.5	62.7	39.8
OX40+CD8+T cell	34.3	59.9	70.4

PBMC, peripheral blood mononuclear cell; Th1, helper T1 cell; Th2, helper T2 cell; Th17, helper T17 cell; Th1/17, helper Th1/17 cell.
The changes in immune cell subsets of PBMC were evaluated at 3 time points: prior to administration of nivolumab; left column, after 1 cycle of treatment; central column, and at the time of developing RS3PE; right column. The edema and joint pain were improved in one week after the administration of prednisolone.

imaging mass cytometry (Hyperion Imaging System, Fluidigm, South San Francisco, CA, USA) showed CD4+ or CD8+ T cells infiltrating the perivascular area and macrophages infiltrating the interstitium of the skin (Figure 2).

Based on these findings, RS3PE as an irAE of nivolumab was diagnosed. Prednisolone (PSL) 15 mg/day (0.3 mg/kg) was administered to treat the RS3PE. After the administration of PSL, the edema of the dorsa of the hands and lower legs improved in one week (Figure 3), and the shoulder joint pain resolved. The serum levels of matrix metalloproteinase 3, vascular endothelial growth factor (VEGF), and interleukin (IL)-6 before PSL administration were 230 ng/mL, 384 pg/mL, and 561.8 pg/mL, respectively. After the administration of PSL, they decreased to 142 ng/mL, 270 pg/mL, and 140.2 pg/mL, respectively. On ultrasonographic examination, improvement in the inflammation of the flexor tendon sheath synovium was observed. The dose of PSL was reduced to 12.5 mg/day after 2 weeks and to 10 mg/day 2 weeks later. However, after the PSL dose reduction, edema and arthralgia of both hands recurred, and the PSL dose was increased to 15 mg/day again. After the dose escalation, joint symptoms and edema improved, and administration of PSL 12.5 mg/day was continued. Nivolumab was administered for a total of 9 cycles, but the CT examination in April 2018 showed an increase in the size of the primary lesion and lymph node lesions, and PD was confirmed. Irinotecan was administered as post-treatment for AGC. After six cycles of chemotherapy, PD was confirmed by upper gastrointestinal endoscopy, but recurrence of RS3PE was not observed. The patient was transitioned to best supportive care.

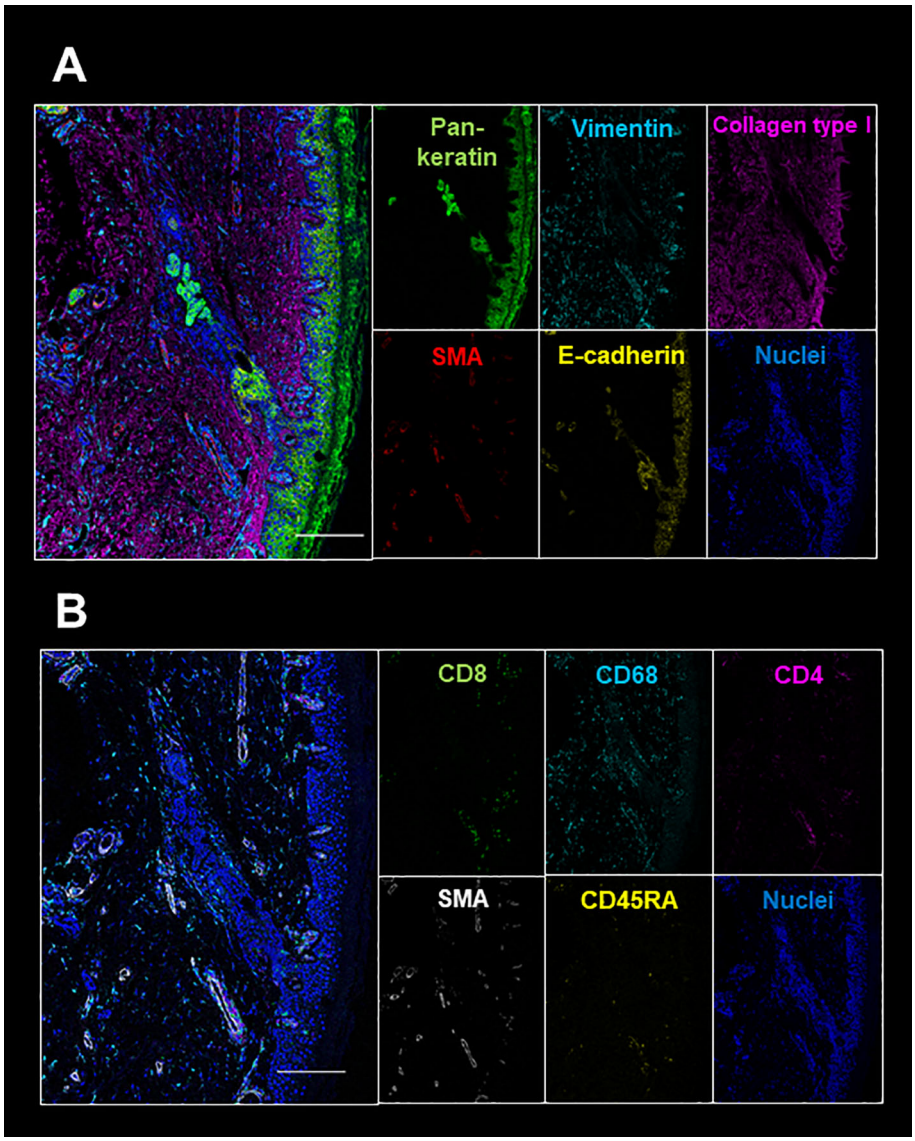

FIGURE 2
Epithelial marker (pan-keratin, E-cadherin), mesenchymal marker (vimentin, SMA), collagen type I **(A)**, and immune cell markers (CD4, CD8, CD45RA, CD68) **(B)** are shown. The white bar indicates 200 μm.

3 Discussion

3.1 Diagnosis of RS3PE

RS3PE syndrome is a disease characterized by edema with bilateral synovitis. It has the following characteristics: (i) better prognosis (remitting); (ii) bilateral symmetry (symmetrical); (iii) negative for rheumatoid factor (seronegative); (iv) acute onset synovitis (synovitis); (v) pitting edema of the dorsa of the hands; (vi) onset in elderly persons; (vii) sudden onset; (viii) no bone erosion; (ix) no pain of the wrists with finger movement restriction; and (x) findings of inflammation (increased CRP or erythrocyte sedimentation rate; ESR) (10). Clinical signs of RS3PE overlap with those of PMR in terms of their response and remission to small doses of steroids. It is in fact difficult to distinguish between PMR and RS3PE because there is no specific test for RS3PE, but RS3PE is characterized by the pitting edema of the dorsa of both hands, and the present patient was diagnosed with RS3PE syndrome.

3.2 Pathology of RS3PE

The pathology of RS3PE is not well elucidated, but it is thought that vascular permeability due to VEGF is associated with the development of edema. It has been reported that the VEGF level in the peripheral blood of RS3PE cases was significantly higher than that of rheumatoid arthritis cases or healthy subjects, and the value was significantly higher than that of healthy subjects, and the VEGF level decreased with steroids (18). In addition, levels of the serum inflammatory cytokine IL-1, IL-6, and TNF-α were elevated, and HLA-B7, HLA-A2, HLA-CW7, and HLA-DQW2 were detected in RS3PE cases. Activated CD4+T cells (CD3+CD4+HLA-DR+),

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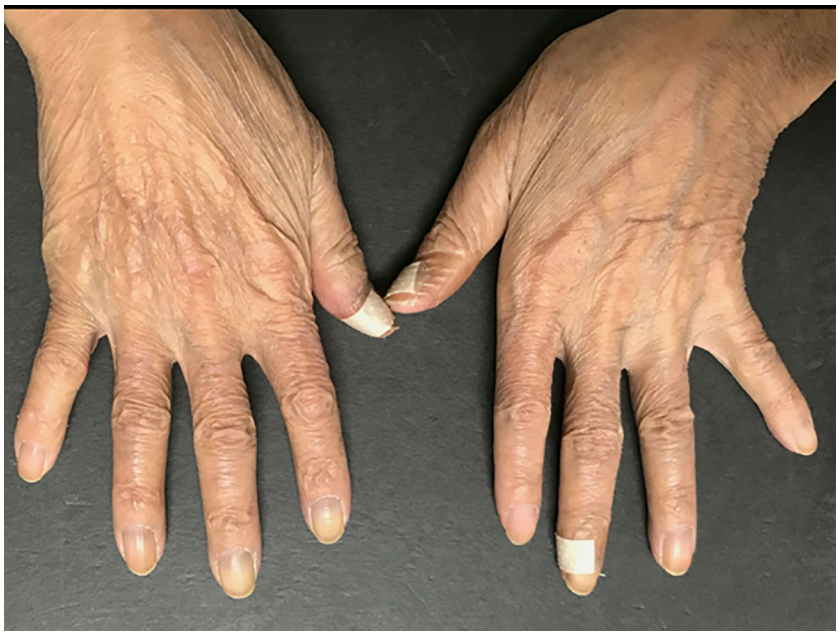

FIGURE 3
After the administration of PSL, the edema of the dorsa of the hands has improved in one week.

helper T1 cells (Th1; CD4+IFN-gamma+IL-4-), and type 1 cytotoxic cells (Tc1; CD8 + IFN-γ + IL-4-) were also detected in the peripheral blood of RS3PE cases (19, 20), suggesting that this disease is associated with autoimmunity. In the present case, the proportion of activated CD4+T cells also increased after administration of nivolumab. Infiltration of macrophages and T lymphocytes is observed in the synovitis of PMR, and it is considered that release of inflammatory cytokines from these cells establishes the pathological condition (21), but the pathology of the synovitis of RS3PE has not been well elucidated. IL-6, MMP3, and VEGF are associated with RS3PE synovitis, and, therefore, their serum concentrations may be useful for evaluating disease activity (18, 22). Serum levels of IL-6, MMP3, and VEGF were also high in the present case. In addition, the serum MMP3 and IL-6 concentrations decreased with steroid administration, indicating the activity of RS3PE. On the other hand, the inflammatory cytokines TNFα and IL-1beta have been reported to be less associated with RS3PE (11), and elevation of these cytokines was not observed in the present case.

3.3 Mechanism of the development of RS3PE

The mechanism of the development of RS3PE remains unknown. However, it has been suggested that a paraneoplastic syndrome or infection is associated with its development (23). It has been reported that parvovirus and *Mycoplasma pneumoniae* infection are triggers of RS3PE (24, 25). In the present case, there was no finding suggestive of the onset of these infections. On the other hand, the present case had AGC, and it is possible that RS3PE developed as a paraneoplastic syndrome. A paraneoplastic syndrome is thought to develop when an immune response to the tumor also occurs in the normal organs of the cancer-bearing host. Although the specific mechanism, such as common antigens, is unknown in RS3PE associated with gastric cancer, a similar mechanism may have existed in the background in the present case as well. RS3PE as a paraneoplastic syndrome can develop before the diagnosis of the tumor and develop relatively early in the clinical course of the cancer (26). The present case showed no symptoms of RS3PE during the course of the first- and second-line chemotherapies, and the first onset of RS3PE was after the administration of nivolumab as the third-line treatment, which was thought to be different from a paraneoplastic syndrome. That is, there was an auto-reactive T cell repertoire that caused RS3PE, and RS3PE did not develop until the second-line treatment, but the peripheral tolerance mechanism was disrupted by nivolumab administration, resulting in RS3PE. In the present case, coinhibitory and costimulatory molecule-positive T cells increased after the administration of nivolumab. It has been reported that chronic antigen stimulation of antigen-specific T cells increased the expression of coinhibitory and costimulatory molecules (17). Expression of these molecules on self-antigen reactive T cells might be enhanced after administration of nivolumab. In so-called isolated RS3PE, which is not paraneoplastic, activation of CD4+ or CD8+ T cells in peripheral blood and enhanced IFN-gamma production from these cells have been observed (20). This also suggests that administration of nivolumab to patients in the pre-clinical stage of RS3PE may induce RS3PE. In this case, pathological analysis for RS3PE as an irAE was performed for the first time. Infiltration of lymphocytes in the perivascular and interstitial layers of the skin was observed, which is consistent

with the finding of isolated RS3PE and supports the possibility that the activation of T cells after administration of nivolumab caused an irAE in skin tissue.

4 Conclusion

This is the first case of a patient with gastric cancer who developed RS3PE after the administration of nivolumab. It was also shown that immune activation by nivolumab is associated with histological findings typical of RS3PE. This is useful for elucidating the mechanism of irAEs with anti-PD-1 treatment.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by ethics committee of Kyushu University Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individuals for the publication of any potentially identifiable images or data included in this article. Written informed consent was obtained from the participant for the publication of this case report.

Author contributions

HO: Data curation, Writing – original draft, Writing – review & editing, Conceptualization, Investigation, Methodology. MK: Writing – review & editing. MU: Writing – review & editing. HA: Writing – review & editing. MI: Writing – review & editing. KT: Writing – review & editing. MA: Writing – review & editing. HN: Writing – review & editing. KA: Writing – review & editing. EB: Conceptualization, Investigation, Writing – original draft, Writing – review & editing.

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Conflict of interest

HA: Speakers' bureau from Ono Pharmaceutical and Bristol-Myers Squibb, KT: Speakers' bureau from Ono Pharmaceutical, MA: Speakers' bureau from Bristol-Myers Squibb, HN: Honoraria from Ono Pharmaceutical and Bristol-Myers Squibb and Speakers' bureau from Ono Pharmaceutical and Bristol-Myers Squibb, KA: Lecture fees from Ono Pharmaceutical and Bristol-Myers Squibb, EB: Honoraria from Ono Pharmaceutical and Bristol-Myers Squibb.

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

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Immune-related adverse events with severe pain and ureteral expansion as the main manifestations: a case report of tislelizumab-induced ureteritis/cystitis and review of the literature

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Immune checkpoint inhibitor (ICI) is an up-to-date therapy for cancer with a promising efficacy, but it may cause unique immune-related adverse events (irAEs). Although irAEs could affect any organ, irAEs-induced whole urinary tract expansion was rarely reported. Herein, we reported a 27-year-old male patient with thymic carcinoma who received the treatment of tislelizumab, paclitaxel albumin and carboplatin. He was hospitalized for severe bellyache and lumbago after 6 courses of treatment. Antibiotic and antispasmodic treatment did not relieve his symptoms. The imaging examinations reported whole urinary tract expansion and cystitis. Therefore, we proposed that the patient's pain was caused by tislelizumab-induced ureteritis/cystitis. After the discontinuation of tislelizumab and the administration of methylprednisolone, his symptoms were markedly alleviated. Herein, we reported a rare case of ICI-induced ureteritis/cystitis in the treatment of thymic cancer and reviewed other cases of immunotherapy-related cystitis and tislelizumab-related adverse events, which will provide a reference for the diagnosis and treatment of ICI-related irAEs.

KEYWORDS

immune-related adverse events, tislelizumab, cystitis, ureteritis, case report

Introduction

Immune checkpoint inhibitor (ICI) is an emerging immunotherapy for cancers. However, since ICI will activate immune responses, it may cause unique immune-related adverse events (irAEs). irAEs can affect different organs and reduce the survival benefit of immunotherapy if untreated (1). In some cases, irAEs will endanger the lives of patients (2, 3).

Tislelizumab (BGB-A317) is a humanized anti-programmed death receptor 1 (PD-1) monoclonal antibody. In clinical studies, tislelizumab has shown promising anti-tumor activity in various solid tumors (4). In these studies, tislelizumab-related adverse events are briefly recorded (5), but few adverse events related to the urinary system are reported. In other case reports, tislelizumab is suggested to induce various immune-related adverse events (2, 3, 6–15). A previous case report indicated that tislizumab could induce ureteritis and cystitis in patients with esophageal cancer (15). However, the chief complaint of the patient in that case report differed from that in our case. Moreover, another PD-1 inhibitor, sintilimab, was reported to cause cystitis and ureteritis (16). Our patient was hospitalized for bellyache with paroxysmal lumbago. He had no obvious symptom of frequent urination, urgency, and pain in urination. The positron emission tomography/computed tomography (PET/CT) and magnetic resonance urography (MRU) scans revealed an expanded whole urinary tract, which is rarely reported. Therefore, this is the first report of ICI-induced ureteritis and cystitis during the treatment of thymic cancer. In addition, we reviewed several cases of immune-induced cystitis (15–25), in which the main manifestations of patients were frequent urination, dysuria, pain on urination, nocturia or incontinence. Hence, our report would provide a reference for the diagnosis and treatment of patients who received ICI and complained of bellyache.

Case presentation

A 27-year-old male patient was admitted to Zhejiang Hospital due to chest pain. He had a history of fatty liver and kidney stones with no history of smoking and drinking. He did not have a medical history of hypertension, diabetes, kidney disease, or hepatitis. His father had hepatitis B, and no family members had a tumor history. The CT showed anterior superior mediastinal and liver mass on March 30, 2022. Pathological results of liver puncture indicated poorly differentiated carcinoma with necrosis. Then the patient was admitted to our hospital on April 9, 2022. The immunohistochemical examination of the liver mass indicated CK (Pan) (+), CD5 (+), CgA (-), SYN (-), P63 (scattered cells+), CD117 (+), GLUT-1 (+), Muc-1 (+), CD3 (-), P40 (scattered cells+), CD56 (-), CK20 (-), Ki67(+≈90%) (Appendix Figure S1), which suggested that it was liver metastasis of thymic carcinoma. The expression of PD-L1 is positive in 70% of tumor cells (clone 22C3, Dako, Glostrup, Denmark). Also, we sequenced the 520 pan-cancer genes in formalin fixation and paraffin embedding specimens (Appendix Table S1). The tumor mutation burden (TMB) was 10.2 Muts/Mb, which was higher than 99% thymic carcinoma. The ratio of mutation at microsatellite site was 1.65% (2/121), which indicated microsatellite stability (MSS). PET/CT showed that the size of the tumor in anterior superior mediastinum was 4.1 cm × 3.5 cm, and standard uptake value (SUV) max was 16.6. The boundaries between the tumor and adjacent superior vena cava, pericardium and mediastinal pleura were not clear (Figure 1A). The tumor had liver (Figure 1B), lymph nodes and bone metastasis. Based on these results, the tumor was staged as pTxN1M1. His performance score (PS) was 1 (PS ranged from 0 to 6, and the lower value indicated better physical condition). The patient began to receive chemo-immunotherapy on April 14, 2022. He was administered with paclitaxel albumin (CSPC, OUYI, Pharmaceutical Co, Ltd) (200 mg at Day 1, Day 8), carboplatin (Bristol-Myers Squibb S.r.l., 0.3 g at Day 1, Day 8) and tislelizumab (Baize'an, BeiGene Ltd., Beijing, China, 200mg at Day 1) for 6 courses. Chest CT and liver MRI showed significant reduction of the tumor and the treatment reached partial response (PR). On August 19, the patient developed bellyache, which was day 7 since the last treatment course. The bellyache last for 2 days, with significant pain on the left side and paroxysmal lumbago and no gross hematuria. The symptom could relieve on itself.

The patient had percussion pain (+) in renal area. Urinalysis showed red blood cells (+++) and white blood cells (++). His white blood cells in routine blood examination was 16.76 (normal 3.5-9.5)×10^9/L. B-ultrasound examination in bladder was normal and that in abdomen showed bilateral kidney stones. The level of glutamic pyruvic transaminase (GPT), serum amylase and serum creatinine was normal. Therefore, we proposed that the patient had a urinary tract infection due to the urinary calculi, and we administered levofloxacin (0.5 g) and phloroglucinol (80 mg) for 3 days. However, his pain did not subside. Initially, the patient had a breakthrough pain once a day, with the Numerical Rating Scale (NRS) score of 7-8. Later, the frequency of pain increased to 3 times a day and the pain was obvious when urinating, based on which we conducted urine culture, but failed to identify any pathogen. PET/CT scan showed that the mass at the right anterior mediastinal was smaller than before (2.3 cm × 2.2 cm vs 4.1 cm × 3.5 cm), and the metabolism was reduced (SUVmax 4.5 vs 16.6). There was no significant increase of 18F-Fluorodeoxyglucose (FDG) fluorodeoxyglucose (FDG) metabolism in metastatic tumor at lymph nodes and liver (Figures 1C, D). Meanwhile, PET/CT revealed, compared to the previous images (Figure 1E), poor bladder filling, slightly thickened bladder wall, slightly enlarged left kidney, increased FDG metabolism in bilateral renal parenchyma, dilated bilateral ureters with smooth excretion, and no obvious ureteral calculus (Figure 1F). Consistent with previous findings, MRU scan showed that bilateral ureteral wall was slightly thickened and whole ureteral fully expanded (Figure 1G). Cystoscopy indicated cystitis (Appendix Figure S2). Based on these results, a multi-disciplinary treatment (MDT) meeting was organized. The cause of pain excluded the urinary infection, tumor metastasis and nephrolithiasis, and the pain was most probably caused by tislelizumab-induced ureteritis/cystitis. The patient was administered with methylprednisolone intravenously, 1 mg/kg once daily and tislelizumab was discontinued. The patient's persistent bellyache and lumbago were basically relieved, the intensity of paroxysmal pain reduced, and the pain after urination improved

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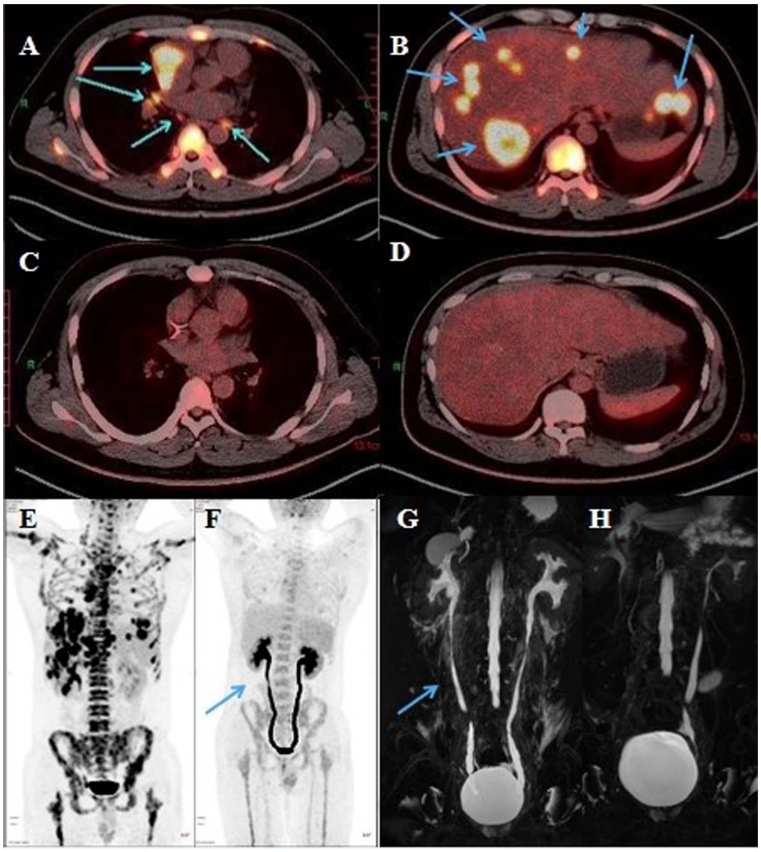

FIGURE 1
Representative radiological images of the patient. **(A)** Mediastinum tumor and **(B)** hepatic metastatic lesion on PET/CT (April 11, 2022). **(C)** Mediastinum tumor and **(D)** hepatic metastatic lesion on PET/CT (August 24, 2022). **(E)** PET/CT (April 11, 2022). **(F)** Bilateral ureters were slightly dilated on PET/CT (blue arrow, August 24, 2022). **(G)** Bilateral ureteral wall was slightly thickened and whole ureteral was full expanded on urinary MRU (blue arrow, August 31, 2022. **(H)** Right ureter expansion was improved, and ureteral exudation was absorbed on urinary MRU (September 22, 2022).

after 3 days of treatment. After he was discharged on September 8, Methylprednisolone (12 mg) was administered orally once daily. During the follow-up, the patient had slightly poor sleep but had no obvious adverse events such as drug allergy, gastric bleeding, and edema, etc. The patient's pain did not recur and the dose of methylprednisolone was decreased gradually. On September 22, the MRU indicated that the dilation of left kidney and right ureter was improved compared to those on August 31, and the around ureteral exudation was absorbed. (Figure 1H). Methylprednisolone was finally discontinued about 40 days after treatment. Because the patient's pain was caused by ICI rather than chemotherapy drugs, the patient received paclitaxel albumin and platinum again on September 22, 2022. No similar pain or urinary symptoms occurred during the follow-up. The timeline of treatment course was summarized in Figure 2.

Discussion and review of the literature

The structure of tislelizumab has been modified to maximally block the binding of PD-1 to programmed death ligand 1 (PD-L1) (4). The binding of Fcγ receptors (FcγRs) will impair the anti-tumor activity of anti-PD-1 antibody (26). Several Fc-hinge regions of tislelizumab has been muted to minimize its binding to FcγRs. Up to now, tislelizumab has been approved for the treatment of various tumors, including classical Hodgkin's lymphoma, urothelium cancer, lung adenocarcinoma, non-squamous cancer, liver cancer, esophageal squamous cancer, nasopharyngeal cancer, advanced colorectal cancer, and solid tumors with microsatellite instability-high (MSI-H) or mismatch repair protein deficiency (dMMR) MSI-H or dMMR in China. However, adverse events may occur during tislelizumab treatment. Existing clinical studies suggested that adverse effects of tislelizumab included anemia, leukopenia, thrombocytopenia, nausea, increased aspartate transaminase (AST), neutropenia, fatigue, decreased appetite, vomiting, musculoskeletal pain, constipation, hypoproteinemia and rash (4, 5). To better understand the adverse events of tislelizumab, we searched for available case reports and reviewed tislelizumab-associated adverse events (Table 1). Although irAEs can affect any organ, tislelizumab-related irAEs in the urinary system are rarely reported. A previous case report indicated that tislizumab could induce ureteritis and cystitis in patients with esophageal cancer (15). However, the chief complaint of the patient in that case report differed from that in our case.

Thymic carcinomas are rare malignancies. For unresectable or metastatic thymic carcinomas, chemotherapy is the standard treatment. ICIs are new drugs with promising efficacies in

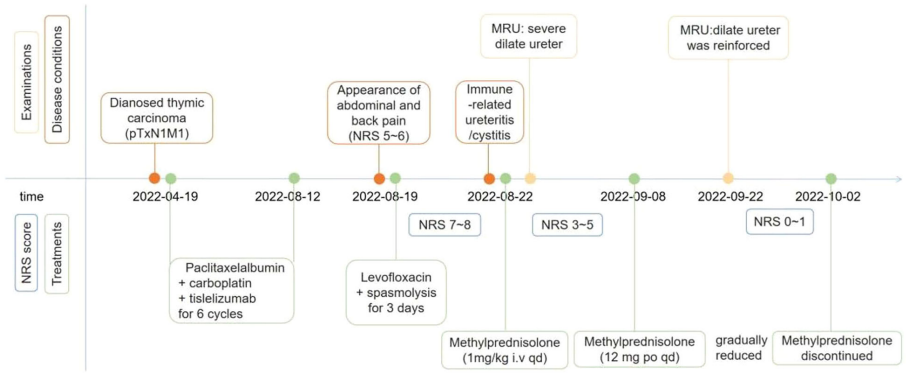

FIGURE 2
The timeline of the patient's symptom, diagnosis and treatments.

cancers. A phase 2 clinical trial of pembrolizumab (27), an anti-PD-1 antibody, IN 40 patients with thymic carcinoma showed that the overall response rate (ORR) was 22.5% and the median progression-free survival (mPFS) was 4.2 months. Moreover, those with high PD-L1 expression benefit more from pembrolizumab treatment. In our case, PD-L1 was 70% positive in patient's tumor cells with a high TMB. A study showed that patients with high TMB and PD-L1 expression had a high rate of durable clinical benefit from ICIs treatment (28). Therefore, our patient is likely to benefit from ICI. However, due to the financial issue, the patient chose another ICI, tislelizumab, for subsequent treatment.

Here, we report a case of tislelizumab-induced ureteritis and cystitis. Up until now, there is no standard for the diagnosis of immune-related cystitis, where cystoscopic biopsy may help. In our case, we suspected that patient's bellyache was caused by kidney stones since abdominal B-ultrasound showed bilateral kidney stones. However, no stone was found on urinary CT, which might be due to the small size of the stone that was not shown on CT. Since small kidney stones rarely caused such severe and long-term pain, we further performed PET/CT and found no tumor metastasis of the urinary system. Nevertheless, ureteral expansion was identified by PET/CT and MRU, and cystoscopy suggested

TABLE 1 Tislelizumab-related adverse events in available case reports.

Malignancies	Age (yr)	Gender	Diagnosis	Cycles	Reference
NSCLC	75	F	Lichen planus pemphigoides	1	Kerkemeyer et al. (6)
NSCLC	73	M	Colitis with Clostridium difficile positive	≈8	Ni et al. (7)
NSCLC	74	M	Nephrotic syndrome, membranous nephropathy	11	Chen et al. (8)
NSCLC	71	M	Severe myositis, myocardial damage, hepatic damage, secondary adrenal insufficiency	1	Deng et al. (3)
AGC	66	F	Pulmonary embolism, deep vein thrombosis	1	Fu et al. (2)
UUC	66	M	Myocarditis	UN	Hu et al. (9)
NSCLC	56	M	Herpetiform pemphigus	6	Zhang et al. (10)
AGC	58	M	Hypothyroidism and adrenal insufficiency	6	Baek et al. (11)
AGC	59	M	Subclinical hypothyroidism and adrenal insufficiency	13	Baek et al. (11)
CHL	25	F	Grade 2 immune-related pneumonitis	2	Zhou et al. (12)
LACRC	65	M	Severe myasthenia gravis, myocarditis, and rhabdomyolysis	1	Wang et al. (13)
CHL	26	F	Tumor flare reaction	4	Zhu et al. (14)
EC	49	F	Ureteritis and cystitis	6	Li et al. (15)
TC	27	M	Ureteritis/cystitis	6	This case

NSCLC, non-small cell lung cancer; UUC, ureteral urothelial cancer; AGC, advanced gastric cancer; LACRC, locally advanced colorectal cancer; CHL, classic Hodgkin lymphoma; EC, esophagus cancer; TC, Thymic carcinoma; UN, unknown; M, male; F, female; mPSL, methylprednisolone; PSL, prednisolone; ≈, about.

cystitis. After excluding kidney stones, tumor invasion, and urinary tract infection, we considered that the patient's pain was caused by immune-related ureteritis and cystitis. After the steroid administration, the pain was markedly alleviated, and the following MRU suggested that ureteral expansion was relieved. Therefore, we confirmed the diagnosis that the patient's pain and ureteral dilatation were caused by tislelizumab-induced ureteritis and cystitis.

irAEs are toxicities caused by non-specific activation of the immune system and can affect almost any organ (29). However, the exact mechanism of irAEs is not clear, which may involve the activation of various inflammatory cells, such as Th17 and other types of cells (29). Other studies indicated that irAEs might occur because of impaired immune tolerance and molecular mimicry (21). Studies had found that PD-L1 was expressed in bladder tissue in patients with severe bladder inflammation. Therefore, it is speculated that PD-1/PD-L1 mAb-induced cytotoxic T-cell activation may simultaneously target at cancer and normal urothelial cells (15). A meta-analysis showed that the incidence of irAEs significantly increased when ICI was combined with chemotherapy (21).

In published clinical trials and case reports, there is rarely report on tislelizumab-induced ureteritis and cystitis. Therefore, we referred to previous case reports on autoimmune cystitis caused by more than tislelizumab (Table 2). Among these cases, 50% (7/14) patients received nivolumab, 14% (2/14) patients received pembrolizumab, 21% (3/14) patients received sintilimab, 7% (1/14) patient received atezolizumab, and 7% (1/14) patient took tislelizumab. Moreover, there is no clear timing for the onset of immune-related cystitis during ICIs treatment. The main manifestations of patients in these case reports were frequent urination, dysuria, urination pain, nocturia, incontinence, or diarrhea. Only two of them had low back pain, and one received tislelizuma (16) and another one received sintilimab (15). As a comparison, the manifestation of our patient was bellyache, which was different from that in these immune-induced cystitis cases. More importantly, the ureter of this patient was expanded. Such cases with irAEs in urinary system and expanded ureter are rarely reported. A case of irAEs induced by tislelizumab exhibited different manifestation. Therefore, this is the first report of autoimmune ureteritis/cystitis in the treatment of thymic cancer. Our case will provide a reference for the diagnosis and treatment of ICI-induced ureteritis and cystitis manifested by bellyache.

This study had some limitations. Firstly, we did not give timely steroids treatment. Since the patient had a history of kidney stone, our primary thought of the patient's pain was consequence of kidney stones. When conventional treatment of antibiotics and antispasmodic treatment failed to relive the pain, additional examination of PET/CT and MRU indicated the ureter expansion and cystoscopy indicated cystitis. Based on these results, we considered the pain was caused by tislelizumab-induced ureteritis/cystitis. Secondly, we did not perform cystoscopy after his pain was alleviated because the patient refused. Otherwise, we cloud better observe the changes in the inner wall of the bladder after discontinuing tislelizumab. Thirdly, the diagnosis of cystitis was made based on cystoscopy examination revealing the inflammatory reaction of the inner wall of the bladder, and we did not perform pathological examination.

In conclusion, we reported a rare case of tislelizumab-induced ureteritis/cystitis mainly presented with severe pain and ureteral

TABLE 2 Clinical information of the case reports of irAEs cystitis.

Malignancies	Age(yr)/sex	Gender	ICIs	Cycles of onset from ICIs	Reference
NSCLC	62	M	Nivolumab	3	Ozaki et al. (17)
NSCLC	50	M	Nivolumab	7	Shimatani et al. (18)
NSCLC	60	M	Nivolumab	12	Shimatani et al. (18)
NSCLC	78	F	Pembrolizumab	6	Ueki et al. (19)
NSCLC	47	M	Nivolumab	18	Yajima et al. (20)
SCLC	51	M	Nivolumab	5	Zhu et al. (21)
NSCLC	53	M	Sintilimab	3	Tu et al. (16)
GC	56	M	Sintilimab	5	Wang et al. (22)
BC	67	F	Atezolizumab	4	Obayashi et al. (23)
NSCLC	56	M	Pembrolizumab	6	He et al. (24)
Primary lung cancer	60	M	Nivolumab	77	Fukunaga et al. (25)
EC	49	F	Tislelizumab	6	Li et al. (15)
GC	62	F	Sintilimab	3	Li et al. (15)
GC	49	F	Nivolumab	2	Li et al. (15)
TC	27	M	Tislelizumab	6	This case

NSCLC, non-small cell lung cancer; GC, gastric cancer; BC, breast cancer; SCLC, small cell lung cancer; TC, Thymic carcinoma; mPSL, methylprednisolone; PSL, prednisolone; M, male; F, female; UN, unknown.

expansion. This case reminds us of the potential risk of urinary system during ICI treatment. Since tislelizumab is currently used in various malignancies, our case will provide a reference for the diagnosis and treatment of ICI-related irAEs.

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 humans were approved by Ethics Committee of Zhejiang Provincial People's Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual for the publication of any potentially identifiable images or data included in this article. Written informed consent was obtained from the participant/patient(s) for the publication of this case report.

Author contributions

QZ designed the case report and drafted the manuscript. ZQ analyzed the patient data and revised the manuscript. PY proposed the concept of this case report. QW and JQ administered the whole course of diagnosis and treatment in this patient. YC analyzed the patient data, and provided significant contributions to the analysis of the patient data. 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.2023.1226993/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Histopathology and immunohistochemistry of the liver biopsy specimens of this patient in April 2022. **(A)** HE staining revealed poorly differentiated carcinomas (20×), IHC staining showed that liver cells were positive for **(B)** CK(Pan) (20×), **(C)** CD5 (20×), **(D)** CD117 (20×), **(E)** GLUT-1 (20×), **(F)** Muc-1 (20×).

SUPPLEMENTARY FIGURE 2
Cystoscope scan (September 1st, 2022).

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Immune checkpoint inhibitor–associated myocarditis: a systematic analysis of case reports

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Background: Immune checkpoint inhibitors (ICIs) therapy can be complicated by their potential cardiovascular toxicities, including myocarditis. Nowadays, no prospective trials have focused on ICI-associated myocarditis optimized management. Available evidence only come from case reports or series. A systematic case reports analysis was conducted to collect and evaluate emerging evidence of ICI-associated myocarditis to provide more information to clinicians.

Methods: We performed a literature search for eligible case reports or series published between January 2018 and May 2023 using the PubMed database. Then, we extracted interesting information via table form. Finally, this study included 113 publications on 106 patients with ICI-associated myocarditis.

Results: Myocarditis was found to be a highly life-threatening disease, with 53.8% of cases. Over half of cases were life-threatening (G4, 23.6%) or severe (G3, 35.8%) and required glucocorticoids. Higher rates of improvement were associated with the best response to ICI for complete response/partial response (72.7% vs. 53.9%), glucocorticoid administration (30% vs. 22%), and discontinuation of ICI (58.8% vs. 32.1%). Consequently, ICI-associated G3–G4 myocarditis should be treated with a combination of discontinuation of ICIs, high-dose glucocorticoids, other drugs, chemical drugs, plasma exchange, and life support. For moderate G1 or G2 cases, discontinuation of ICIs and regular-dose glucocorticoids should be considered.

Conclusion: Once full recovery or improvement was achieved; glucocorticoids can be administered at low doses or stopped. Notably, re-challenge with ICIs appears feasible after resolution or meaningful improvement of myocarditis.

KEYWORDS

immune checkpoint inhibitor, ICI-associated myocarditis, glucocorticoids, cardiovascular toxicities, case reports and series, immune-related adverse events

Introduction

Over the past few decades, immunotherapy has revolutionized cancer treatment and has become the fourth antitumor modality after surgery, radiotherapy, and chemotherapy (1). As the frontier of cancer immunotherapy, immune checkpoint inhibitors (ICIs) have led to considerable clinical breakthroughs and extended survival rates across in a wide range of tumor types (2). ICIs are key negative regulators of antitumor immunity monoclonal antibodies, which can block immune checkpoint proteins including programmed cell death protein 1 (PD-1), programmed death ligand 1 (PD-L1), cytotoxic T-lymphocyte antigen 4 (CTLA-4), and lymphocyte activation gene 3 (LAG-3) (3). Approximately 50% of patients with cancer are eligible for ICI therapy, and a larger number of patients achieve long-term clinical responses (4). As of May 2023, 11 ICIs have been approved for marketing by the United States Food and Drug Administration (Table 1). The increasing number of annual clinical trials reflects the prominence of ICIs in cancer treatment (5).

Given that ICIs can inhibit T cells and activate immune responses, they can cause immune-related adverse events (irAEs) in any organ (6). Although any organ system can be implicated by ICI-associated irAEs, ICI-associated myocarditis has aroused as a rare and often fatal adverse event (7). Other cardiovascular toxicities include vasculitis, pericarditis, and arrhythmias (8). Timely diagnosis and proper treatment of cardiovascular irAEs, especially ICI-associated myocarditis, are clinically challenging (9). Although uncommon (<1% of patients with cancer are treated with ICIs) (10, 11), the morbidity of ICI-associated myocarditis is probably underestimated with inconsistent screening criteria and nonspecific symptoms. In clinical practice, cardiovascular irAEs may manifest occasionally; this view may attribute to the poor understanding of the disease and the failure to recognize early symptoms (12). However, ICI-associated myocarditis has a high death rate, ranging from approximately 20% to 50%, according to retrospective studies (11, 13). Inconsistent morbidity and mortality of ICI-associated myocarditis reflect an unmet clinical need; therefore, understanding the precise mechanisms of pathogenesis and having more clinical information of ICI-associated myocarditis are crucial for timely diagnosis and treatment.

Recently, some authoritative recommendations have been specifically established for the diagnosis and treatment of ICI-associated myocarditis, such as 2022 ESC Guidelines on cardio-oncology (14), Myocarditis in the Setting of Cancer Therapeutics (15). Besides, management of ICI-associated myocarditis can be found in the *Guidelines of Immune-Related Adverse Events in Patients Treated with Immune Checkpoint Inhibitor Therapy* published by the American Society of Clinical Oncology (16). However, no prospective trials have focused on ICI-associated myocarditis' optimized management, and the available evidence was case reports or series. With the high mortality of ICI-associated myocarditis, a timely diagnosis and management is necessary to decrease the death rate and increase the application scope of ICIs in cancer patients. Therefore, we conducted a systematic analysis of case reports for the purpose of collecting and evaluating emerging evidence of ICI-associated myocarditis to provide more information to clinicians.

Abbreviations: ATG, anti-thymocyte globulin; ECG, electrocardiogram; ICI, immune checkpoint inhibitor; CR/PR, complete response/partial response; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1; CTLA-4, cytotoxic T-lymphocyte antigen 4; LAG-3, lymphocyte activation gene 3; MRI, magnetic resonance imaging; irAEs, immune-related adverse events; SD, stable disease; PG, progressive disease.

Materials and methods

Search strategy

We first performed a literature search for eligible case reports or series published between January 2018 and May 2023 using the PubMed database. Then, we carried out a further search using the following combination of terms: ('checkpoint inhibitors' OR 'checkpoint inhibition' OR 'checkpoint blockade' OR 'PD1' OR 'PDL1' OR 'CTLA4' OR 'sintilimab' OR 'pembrolizumab' OR 'camrelizumab' OR 'nivolumab' OR 'tremelimumab' OR 'ipilimumab' OR 'atezolizumab') AND ('carditis' OR 'myocarditides' OR 'myocarditis' OR 'cardiac adverse event' OR 'cardiac side-effect' OR 'cardiac toxicity' OR 'cardiac complication' OR 'cardiac irAE' OR 'Heart Failure'). A detailed flowchart of the study is shown in Figure 1.

Eligibility criteria

Case reports were selected by preliminarily assessment of titles and abstracts. For searching additional qualifying papers, the reference lists of the included literature were curated manually. The inclusion criteria were as follows: (1) studies on ICI-associated myocarditis; (2) full-text available; (3) published papers; and (4) case reports or case series. The exclusion criteria were as follows: (1) articles, reviews, commentaries, and meta-analyses; (2) articles not written in English; (3) studies on cancer agents other than ICIs; and (4) no myocarditis studies.

Study selection and quality assessment

All studies were independently evaluated through the Rayyan platform by screening titles and abstracts with three individuals in parallel (17). The authors assessed the studies based on the aforementioned eligibility criteria and any disagreements were resolved by a third reviewer. The quality assessment of this article of case reports was conducted by previous study. Sufficient quality was determined if five of the eight evaluation criteria were met and all authors agreed that the study could be included.

Data extraction

For the included studies, three authors manually retrieved and extracted the related data. Details were extracted from each case

TABLE 1 FDA-approved immune checkpoint inhibitors by 2023.

Types of ICIs	Drug	Approval	Sum of Indications	Indications
Anti-PD-1	Pembrolizumab	2014	18	Melanoma, Non-Small Cell Lung Cancer, Head and Neck Squamous Cell Cancer, Classical Hodgkin Lymphoma, Primary Mediastinal Large B-Cell Lymphoma, Urothelial Carcinoma, Microsatellite Instability-High or Mismatch Repair Deficient Cancer, Microsatellite Instability-High or Mismatch Repair Deficient Colorectal Cancer, Gastric Cancer, Esophageal Cancer, Cervical Cancer, Hepatocellular Carcinoma, Merkel Cell Carcinoma, Renal Cell Carcinoma, Renal Cell Carcinoma, Tumor Mutational Burden-High Cancer, Cutaneous Squamous Cell Carcinoma, Triple-Negative Breast Cancer.
	Nivolumab	2014	11	Melanoma, Non-Small Cell Lung Cancer, Malignant Pleural Mesothelioma, Renal Cell Carcinoma, Classical Hodgkin Lymphoma, Squamous Cell Carcinoma of the Head and Neck, Urothelial Carcinoma, Colorectal Cancer, Hepatocellular Carcinoma, Esophageal Cancer, Gastric Cancer, Gastroesophageal Junction Cancer and Esophageal Adenocarcinoma
	Cemiplimab	2018	3	Cutaneous Squamous Cell Carcinoma, Basal Cell Carcinom, Non-Small Cell Lung Cancer
	Dostarlimab	2021	2	Endometrial Cancer, Solid Tumors
	Retifanlimab	2023	1	Metastatic or recurrent locally advanced merkel cell carcinoma
Anti-PD-L1	Atezolizumab	2016	6	Urothelial Carcinoma, Non-Small Cell Lung Cancer, Small Cell Lung Cancer, Hepatocellular Carcinoma, Melanoma, Alveolar Soft Part Sarcoma
	Avelumab	2017	3	Merkel Cell Carcinoma, Urothelial Carcinoma, Renal Cell Carcinoma
	Durvalumab	2017	4	Non-Small Cell Lung Cancer, Small Cell Lung Cancer, Biliary Tract Cancer, Unresectable Hepatocellular Carcinoma
Anti-CTLA-4	Ipilimumab	2011	7	Melanoma, Renal Cell Carcinoma, Colorectal Cancer, Hepatocellular Carcinoma, Non-Small Cell Lung Cancer, Malignant Pleural Mesothelioma, Esophageal Cancer
	Tremelimumab	2022	2	Hepatocellular Carcinoma, Non-Small Cell Lung Cancer
Anti-LAG-3	Relatlimab	2022	1	Unresectable or Metastatic Melanoma

PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; LAG-3, lymphocyte activation gene 3.

report as follows: reference information (reference tittle, first author, year); basic patient information (sex, age, past medical history, cancer type, and cancer stage); ICI treatment information (ICI treatment type, therapy line, and ICI drug name); ICI-associated myocarditis information (time to onset, myocarditis diagnosis and staging, myocarditis symptoms, best response to ICI, and prognosis); and other relevant information (ICI discontinuation type, ICI-associated myocarditis treatment strategies, treatment outcome, ICI re-challenge, ICI-associated myocarditis recurrence, and associated irAEs). All data were extracted and compiled into an online Excel file with accessible permissions to all the authors.

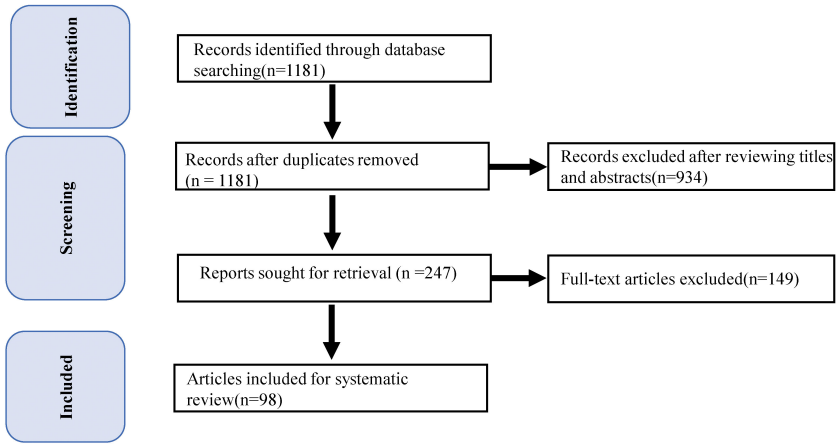

FIGURE 1
PRISMA Participant Flow Diagram. Flow diagram depicts the: 1) methods and results for our strategies; and 2) results of record screening and inclusion in the systematic review of case reports and series.

The data extracted from each article are summarized and presented in Supplementary Material. The cases will be described narratively, combine and highlight the similarities between them, if possible, draw conclusions. Considering the abstractibility of this article and the small cases loads, we used descriptive statistics to exhibit the demographic and clinical characteristics of these cases. Continuous variables were reported by means, and dichotomous variables were characterized by frequencies and percentages.

Results

The search strategy identified 1181 records, all of which were screened based on titles and abstracts. Ultimately, 98 publications were selected, including 116 cases of ICI-associated myocarditis. A descriptive summary of these 106 cases is presented in Supplementary Material.

Table 2 provides a summary of the main characteristics of patients. The median time to onset of myocarditis was 22.5 days (IQR 16–52) following the initiation of ICI treatment. However, some cases of early toxicity and late toxicity are noted within the first week (18, 19) and after ≥1 year of ICI treatment (20), respectively.

Of the 116 included patients, the majority were male (66.4%) and received PD-1/PD-L1 inhibitors as monotherapy (75.0%). The three most common primary tumors were lung cancer (26.7%), melanoma (19.0%), and esophageal/gastric cancer (6.9%). In addition, ICIs are usually used as first/second-line (75.0%) treatment. Following ICI treatment, most patients exhibited stable disease (SD, 37.1%) or progressive disease (PD, 34.5%). ICI-associated myocarditis was severe or life-threatening (G3 or G4) in most cases (60.4%), with only a small proportion of patients (6.0%) experiencing grade 1 myocarditis. Given that myocarditis is a fatal adverse event, the mortality rate was 47.4% among 116 cases. Only 26 cases of recovery (22.4%) and 35 cases of improvement (30.2%) related to this condition were reported. We also summarized the main characteristics of 11 Cases of ICI-associated myocarditis in cancer patients treated and rechallenge with ICIs (Table 3).

Single glucocorticoid agents were administered to 43.1% of the patients, whereas most patients (56.9%) received a combination of glucocorticoids and other therapies. Methylprednisolone was the most frequently administered glucocorticoid, accounting for 43.1% of the cases. The most common methylprednisolone schedule was 1–2 mg/kg/day. Additionally, combination strategies involving glucocorticoids with other therapeutic agents, such as chemical drugs (10.3%), biologics (2.6%), life support (5.2%), immunoglobulins (8.6%), or plasma exchange (5.2%), have also been applied to treat patients with myocarditis of different severities. At the onset of myocarditis, 12 patients (10.3%) had already completed all ICI treatments, and only 14 (12.1%) continued ICIs, whereas 59 (50.9%) discontinued ICIs temporarily (11 patients) or permanently (48 patients). After re-challenge with ICIs, only one of the 11 patients experienced myocarditis recurrence.

TABLE 2 Characteristics of patients with myocarditis under ICI treatment.

N=116	
Gender	**n (%)**
Male	77(66.4)
Female	39(33.6)
Primary cancer	**n (%)**
Lung Cancer	31(26.7)
Melanoma	22(19.0)
Esophageal/gastric cancer	8(6.9)
Thymoma	8(6.9)
Uroepithelial carcinoma	6(5.2)
Kidney Cancer	9(7.8)
Liver Cancer	4(3.4)
Breast Cancer	3(2.6)
Tissue, liposarcoma	3(2.6)
Bone/spinal cord tumor	3(2.6)
Bladder Cancer	2(1.7)
Gallbladder Cancer	3(2.6)
Cervical Cancer	2(1.9)
Mesothelioma	2(1.9)
Colorectal cancer	3(2.6)
Nasopharynx, skin and others	7(6.0)
ICI	**n (%)**
Pembrolizumab (PD-1)	32(30.2)
Nivolumab (PD-1)	25(23.6)
Nivolumab+Ipilimumab (PD-1+CTLA-4)	21(19.8)
Camrelizumab (PD-1)	6(5.7)
Sindilimab (PD-1)	10(9.4)
Durvalumab (PD-L1)	2(1.9)
Sindilimab+Anlotinib (PD-1+TKI)	1(0.9)
Sindilimab+Lenvatinib (PD-1+ RTK)	1(0.9)
Camrelizumab+Bevacizumab (PD-1+VEGF)	1(0.9)
Cemiplimab (PD-1)	1(0.9)
Durvalumab+Tremelimumab (PD-L1+CTLA-4)	1(0.9)
Toripalimab (PD-1)	1(0.9)
NA	4(3.8)
Median time of onset, day (min–max) [IQR]	22.5(3-275) [16-52]
Best response to ICI	**n (%)**
CR	6(5.2)
PR	6(5.2)

(Continued)

10.3389/fimmu.2023.1275254

TABLE 2 Continued

N=116	
SD	43(37.1)
PD	40(34.5)
NA	21(18.1)
Grading of Myocarditis	**n (%)**
G1	7(6.0)
G2	39(33.6)
G3	43(37.1)
G4	27(23.3)
Treatment	**n (%)**
Glucocorticoids	50(43.1)
Glucocorticoids+Chemical drugs	12(10.3)
Glucocorticoids+ Immunoglobulins	10(8.6)
Glucocorticoids+Life-Support	6(5.2)
Glucocorticoids+Chemical drugs+Immunoglobulins	6(5.2)
Glucocorticoids+Immunoglobulins+Life-Support	4(3.4)
Glucocorticoids+Biologics	3(2.6)
Glucocorticoids+Plasma exchange	6(5.2)
Glucocorticoids+Chemical drugs+Plasma exchange	3(2.6)
Glucocorticoids+Immunoglobulins+Plasma exchange	4(3.4)
Glucocorticoids+Chemical drugs+Life-Support	3(2.6)
Glucocorticoids+Immunoglobulins+Plasma exchange+Life-Support	2(1.7)
Glucocorticoids+Chemical drugs+Biologics+Life-Support	1(0.9)
Glucocorticoids+Immunoglobulins+Biologics	1(0.9)
Glucocorticoids+Biologics+Life-Support	1(0.9)
Glucocorticoids+Plasma exchange+Life-Support	1(0.9)
Immunoglobulins+Plasma exchange	1(0.9)
Glucocorticoids+Chemical drugs+ Biologics	1(0.9)
Glucocorticoids+Biologics+Chemical drugs	1(0.9)
Outcome	**n (%)**
Recovery	26(22.4)
Improvement	35(30.2)
Death	55(47.4)
Management of ICI	**n (%)**
Treatment already completed at the onset	12(10.3)
Continued	14(12.1)
Temporarily discontinued, then restarted	11(9.5)
Permanently discontinued	48(41.4)
Not reported	31(26.7)

(Continued)

TABLE 2 Continued

N=116	
ICI treatment line	**n (%)**
First-line	33(28.4)
Second-line	54(46.6)
Third-line	8(6.9)
Fourth-line	1(0.9)
Multi-line	1(0.9)
NA	19(16.4)
Associated irAEs	**n (%)**
Yes	64(55.2)
No	52(44.8)
Response to myocarditis treatment	**n (%)**
Improved	50(43.1)
Not improved	40(34.5)
NA	36(31.0)

Patients with complete response/partial response (CR/PR) usually exhibited a higher improvement rate (83.3%) than patients with SD/PD (55.4%). Of the 116 cases included, myocarditis development was followed by continuation of ICIs in 14 (continued after evaluation) and 11 (temporarily discontinued, then restarted) cases, corresponding to an oncologic efficacy of CR (4), PD (8), PR (2), SD (7), NA (4), and a favorable outcome of 52% (13/25) comparable to the outcome of overall ICI treatment of healing and improvement (52.6%). The occurrence of myocarditis did not affect the efficacy of immunosuppressive therapy. In clinical practice, physicians need to carefully and adequately assess the benefit-risk ratio of patients before initiating ICI therapy and after myocarditis before deciding whether to rechallenge ICI. Patients with myocarditis treated with glucocorticoids had a better improvement rate (62.0%) than those who did not receive hormones. Notably, compared with ≤G3 myocarditis patients, G4 patients have higher improvement rate at 59.3%, although these associations were not statistically significant (Table 4).

Discussion

The largest number of published case reports on myocarditis in patients with cancer treated with ICIs were included and analyzed in this article. We presented the main characteristics of the 106 patients and found associations between some patient characteristics and myocarditis outcomes.

Based on our results, male sex, lung cancer, melanoma, and treatment with anti-PD-1, anti-PD-L1, or anti-PD-1 in combination with CTLA-4 may increase the risk of ICI-associated myocarditis. Previous studies have suggested that the combination of anti-CTLA-4 and anti-PD-1 is one of the strongest risk factors for

TABLE 3 11 Cases of ICI-associated myocarditis in cancer patients treated and rechallenge with ICIs.

Author, year	Age, sex	Tumor type	ICI Type	ICI target	myocarditis onset time	myocarditis diagnosis	ICI-related myocarditis Grade	myocarditis symptom	Tumor pro-gression	outcome	Treatment to death time	ICI Discontinu-ation	Treatment method	myocarditis Outcome
Shindo, 2022 (21)	79, Male	Stomach Cancer	PD-1	Nivolumab	11days	Confirmation of diagnosis (2)	Grade 2	Muscle weakness of lower limbs appeared in 11 days	PD	Death by disease progression	2 months	temporarily discontinued	glucocorticoid	Recovery/NA
Gallegos, 2019 (22)	47, Female	Metastatic melanoma	CTLA-4+PD-1	ipilimumab and nivolumab	120days	Confirmation of diagnosis (2)	Grade 4	Low plasma replacement pressure, ventricular tachycardia, pulmonary edema	PD	Death	7 days	temporarily discontinued	glucocorticoid	Death/7 days
Shen, 2021 (23)	53, Female	Thymoma	PD-1	Pembrolizumab (200 mg)	21 days	Confirmation of diagnosis (2)	Grade 2	Cough, chest tightness, muscle weakness, fatigue	PD	Recovery	NA	temporarily discontinued	glucocorticoid	Recovery/6 months
Kee, 2022 (24)	69, Male	Lung cancer	PD-1	Pembrolizumab	23days	Suspected diagnosis (3)	G3	exertional dyspnea and orthopnea,left eye ptosis	SD	death	163days	temporarily discontinued	Glucocorticoids +Chemical drugs +Life-Support +Immunoglobulins	Death/ 163days
Zhang, 2022 (25)	68, Female	thymoma	PD-1	Camrelizumab (200 mg, 1/21d)	11days	Suspected diagnosis (3)	G3	dyspnea, fatigue, and poor appetite,palpitation, and poor appetite	SD	death	5days	temporarily discontinued	glucocorticoid+ immunoglobulin +chemical drug	Death/5days
Wintersperger, 2022 (26)	52, Male	Melanoma	PD-L1	PD-L1	21days	Confirmation of diagnosis (2)	Grade 3	Weakness, shortness of breath	SD	Improved	NA	temporarily discontinued	Glucocorticoids + biologics + chemical drugs	Recovery/ 44days
Lie, 2020 (27)	79, Male	Malignant pleural mesothelioma (MPM)	PD-1	Nivolumab (3 mg/kg)	42days	Confirmation of diagnosis (2)	G3	proximal limb and truncal weakness, dyspnea and generalized fatigue	CR	recovery	NA	temporarily discontinued	Glucocorticoids +Chemical drugs	Recovery/ 90days
Bawek, 2021 (28)	68, Male	melanoma	PD-1	Nivolumab	21days	Possible diagnosis (3)	G2	shortness of breath, intermittent palpitations, dizziness, and nausea	SD	death	14days	temporarily discontinued	glucocorticoid	Death/14 days
Delombaerde, 2022 (29)	69, Male	Metastatic bile duct cancer	PD-1	nivolumab (3 mg/kg) and ipilimumab (1 mg/kg)	21days	Suspected diagnosis (3)	Grade 2	Episodes of low retrosternal epigastric pain without dyspnea, palpitations, nausea, or stool changes after 1 d	SD	Improved	NA	temporarily discontinued	glucocorticoid	Improved/2 weeks
Zhou, 2022 (30)	67, Male	Squamous cell carcinoma of the lung	PD-L1	Durvalumab	7 days	Possible diagnosis (3)	Grade 3	Fever, breathing difficulties	PD	Improved	7 days	temporarily discontinued	Glucocorticoids +Chemical drugs	Recovery/7 days
Hardy, 2020 (31)	81, Male	RCC metastasis	CTLA-4+PD-1	Iipilimumab and nivolumab	21 days	Probable diagnosis (3)	G4	fatigue, decreased appetite, and weight loss.	NA	Death	2days	temporarily discontinued	Glucocorticoids+ plasma exchange	Death/2 days

10.3389/fimmu.2023.1275254

TABLE 4 Association between characteristics of patients and myocarditis outcome.

Characteristics	Outcome	P
Grade at the onset	Improvement rate, % (n/N)	
G4(N=27)	59.3(16/27)	0.2637
≤G3(N=89)	50.6(45/89)	
Best response to ICI	Improvement rate, % (n/N)	
CR/PR(N=12)	83.3(10/12)	0.1140
SD/PD(N=83)	55.4(46/83)	
Glucocorticoids	Improvement rate, % (n/N)	
Yes (N=50)	62.0(31/50)	0.1348
No(N=66)	45.5(30/66)	
Glucocorticoids+Chemical drugs	Improvement rate, % (n/N)	
Yes (N=12)	50.0(6/12)	>0.9999
No(N=104)	52.9(55/104)	
Glucocorticoids+ Immunoglobulins	Improvement rate, % (n/N)	
Yes(N=10)	70(7/10)	0.3287
No(N=106)	50.9(54/106)	
Discontinuation of ICI	Improvement rate, % (n/N)	
YES(N=59)	61.0(36/59)	0.0575
No(N=26)	34.6(9/26)	
Rechallenge of ICI	Recurrence rate, % (n/N)	
YES(N=11)	22.2(2/9)	0.0226
No(N=48)	0(0/48)	

ICI-associated myocarditis. The pharmacovigilance data indicated that a 4.74-fold higher risk of myocarditis than nivolumab alone (32). Our research showed that patients receiving anti-CTLA-4 and anti-PD-1 antibodies may exhibit a higher grade of myocarditis, with 46% incidence of grade 4 myocarditis. Another large retrospective pharmacovigilance study revealed that patients with myocarditis are more often male (66%), having melanoma (40.7%) or lung cancer (32%), and are treated with anti-PD-1/PD-L1 as a single agent (69%) (12). Consistent with these data, patients included in our article were mostly male patients receiving anti-PD-1/PD-L1 antibodies for melanoma or lung cancer. Genetic variations, including somatic or germline tumors, may also contribute (33). Furthermore, clinical trials involving a large number of patients are required to identify predisposing factors for myocarditis and other ICI-associated cardiovascular toxicities.

The exact incidence of myocarditis in patients with cancer treated with ICIs remains unknown. ICI-based cancer trials in the early time did not prospectively screen for myocarditis (34). Current investigations have reported that the incidence rates range from 0.1% to 1.14% across different series (13, 32). This broad range may be attributed to heterogeneity, such as the different grades of severity of the cases and the diverse distribution of potential risk factors for ICI-associated myocarditis (35). In addition, because of the difficulty of myocarditis diagnosis cases in these trials might have been missed. Overall, the true incidence of ICI-associated myocarditis may be higher, and further prospective trials should focus on this issue.

ICI-associated myocarditis represents a clinically unmet problem because it may be fatal. The mortality rates range from approximately 35.8%, as reported in our analysis, to >50%, as reported in a previous study (13). To date, no international consensus has been reached covering ICI-associated myocarditis screening, surveillance, prevention, and treatment. The diagnosis of myocarditis can be challenging in clinical settings, particularly in patients receiving ICIs. In current clinical practice, ICI-associated myocarditis is often a multipronged diagnosis of exclusion, ruling out other causes of symptomatology (for example, cancer progression and acute coronary syndrome), and includes a comprehensive analysis of cardiac imaging, biomarker tests, and endomyocardial biopsy (36). Based on a multicenter study from American College of Cardiology (13), Mahmood et al. (13) proposed that the traditional diagnostic pathway of is the observation of new-onset cardiovascular symptoms in patients receiving ICI therapy, further laboratory and imaging tests, and medical consultations, ultimately leading to a diagnosis of ICI-associated myocarditis. Another expert guideline from European Society of Cardiology (14) indicated that the initial diagnosis of ICI-associated myocarditis relies on the identification of aberrant cardiovascular symptoms, a recent elevation in troponin levels, the presence of new electrocardiogram (ECG) abnormalities, and urgent cardiovascular imaging to other causes of myocardial injury, such as acute coronary syndrome. In fact, most patients exhibit clinical symptoms suggestive of ICI-associated myocarditis, elevated troponin levels, and/or an abnormal baseline ECG (37). However, increased serum troponin concentrations are difficult to interpret in asymptomatic patients, which highlighted improved predictive biomarkers are needed. Cardiac magnetic resonance imaging (MRI) can be used for further diagnosis (38). In clinical practice, an endomyocardial biopsy has traditionally been regarded as the gold standard for myocarditis diagnosis (39). The histopathological characteristics of ICI-associated myocarditis involve infiltration of T lymphocytes (both CD4+ and CD8+), macrophages, and myocyte death, whereas B lymphocytes are not observed (32). However, as an endomyocardial biopsy is an invasive examination, it poses a psychological burden on patients. In the future, prospective multi-institutional studies are needed to explore effective non-invasive examinations, such as predictive biomarkers and medical imaging, for the screening and surveillance of patients.

The clinical implications of ICI-associated myocarditis vary among studies. Patients with fulminant myocarditis exhibit early symptoms after ICI treatment, including arrhythmias/conduction disturbances, dyspnea, concomitant skeletal myositis, and myasthenia gravis (12). This was consistent with our results which showed that dyspnea was found in 31% of patients. Another study highlighted that the concomitant presence of skeletal myositis and myasthenia gravis following after ICI treatment should increase awareness of myocarditis (40). Our research indicated that 20% of patients with myocarditis also exhibited skeletal myositis or myasthenia gravis. In contrast to fulminant cases, “smoldering” cases of ICI-associated myocarditis

have also been documented (35, 41). However, no studies have revealed the long-term consequences of ICI-associated myocarditis. Therefore, given the growing number of cancer survivors receiving ICIs, understanding the long-term cardiovascular effects of ICIs is a future challenge for oncologists and cardiologists.

Treatments for ICI-associated myocarditis have been largely extrapolated from amount of irAEs therapies, including cessation of ICIs, glucocorticoids, chemical drugs, and supportive management (42). For myocarditis, higher initial steroid doses (e.g., intravenous methylprednisolone, 1 g/day) have been suggested (43). In the present review, almost all patients (98.2%) received glucocorticoids and achieved a 15.1% recovery rate and a 35.8% improvement rate, suggesting that glucocorticoids are the cornerstone of ICI-associated myocarditis treatment. Nonetheless, the findings of our analysis revealed that the mortality was substantial (50.8%). In addition to glucocorticoids, various case reports have demonstrated the efficacy of other medications such as tacrolimus (44), mycophenolate mofetil (45), abatacept (46), and alemtuzumab (47). Although these treatments are classified as immunosuppressive modalities, their specific mechanisms of action differ (48). For example, abatacept is a soluble protein composed of the CTLA-4 extracellular domain fused to the Fc region of IgG, which limits the costimulatory signals of T cells (49). Wei et al. explored whether abatacept could ameliorate the disease progression of ICI-associated myocarditis in a mouse model and mitigate its fulminant course in patients (50). Further it is necessary of prospective clinical trials to compare single or combination efficacy with that of other therapies.

Considering steroids as the main treatment for immune myocarditis, we also summarized new immune checkpoint inhibition into the biologic agent category in Table 2, including six case-use reports of infliximab, one case of anti-thymocyte globulin (ATG), and one case of abatacept. Two studies reported nonsignificant improvement in symptoms related to myocarditis with infliximab (51, 52), while three cases reported a worsening manifestation of symptoms related to myocarditis with infliximab (53–55), and another study reported the use of infliximab but not describing the results (26). One study found the use of ATG was suspended due to poor patient status (56), and another study reported the myocarditis symptoms were improved with the use of Abatacept (57). Although new immune checkpoint inhibitors have been recommended as second-line therapy for immune myocarditis after steroid resistance, and although this study summarized case reports on immune myocarditis in the last five years, there is uncertainty about the efficacy of biologics such as tumor necrosis factor-α antagonists, ATG, and abatacept in actual case reports due to the lack of prospective studies, and this may be related to our limitations of the collected cases.

The guidelines of the American Society of Clinical Oncology for the management of irAEs in patients treated with ICIs (42) recommend the early use of high doses of glucocorticoids (e.g., methylprednisolone 1 g/day) and a combination of mycophenolate, antithymocyte globulin, or infliximab for the treatment of refractory and recurrent myocarditis. Conversely, although some experts have advocated TNF-α antagonists (such as infliximab) for ICI-associated myocarditis, concerns have been raised regarding their application in patients with heart failure (58).

Furthermore, we also compared our results with current known cohorts of ICI-associated myocarditis (11–13, 59) (Table 5). Our results were almost consistent with other four cohorts. Based on the known cohorts, the incidence rate of ICI-associated myocarditis is ranged from 0.39%-1.14%, representing a small entity of ICI-associated adverse events. However, the fatal rate is as high as 39.7%-50%, and our results indicate the fatal rate is 47.4%, indicating the unmet clinical need of ICI-associated myocarditis. Besides, the combination of anti-PD-1/PD-L1 and anti-CTLA-4 seems to cause higher fatal rate compared with single use of ICIs. Therefore, in clinical practice, physicians need to carefully and adequately assess the benefit-risk ratio of patients before initiating ICI therapy and after myocarditis before deciding whether to rechallenge ICI.

The exact mechanism of the pathogenesis of ICI-associated myocarditis remains unclear, and some concerns should be addressed in future studies (60) (Figure 2). First, we precisely determined the incidence of ICI-associated myocarditis. The potential lethality of cardiotoxicity limits the clinical application of ICIs. Given the apparent low frequency (<1%) of ICI-associated myocarditis, one would not anticipate this possibility, if not for the high death rate (35.8%, as reported in our analysis) associated with this adverse event. The inconsistent morbidity and mortality rates

TABLE 5 The comparison of this study with current known cohort.

	Wang's study (59)	Moslehi's study (11)	Mahmood's study (13)	Salem's study (12)	Our study
Incidence rate	131(0.42%) of 31,059 cases	NA	11(1.14%) of 964 cases	122(0.39%) of 31,321 cases	NA
Timing (median, range)	32 days (3-355)	27 days (5-155)	34 days (21-75)	30 days (1-240)	22.5 days (3-275)
Fatality rate	52 (39.7%) of 131 cases	46 (46%) of 101 cases	NA	61 (50%) of 122 cases	55 (47.4%) of 116 cases
Anti–PD-1/PD-L1 deaths	27 (8%) of 333 cases	22 (36%) of 61 cases	NA	40 (44.4%) of 90 cases	42 (52.5%) of 80 cases
Anti–CTLA-4 deaths	3 (2%) of 193 cases	3 (60%) of 5 cases	NA	NA	NA
Combination PD-1/CTLA-4 deaths	22 [25%] of 87 cases	18(67%) of 27 cases	NA	21 (65.6%) of 32 cases	12 (54.5%) of 22 cases

10.3389/fimmu.2023.1275254

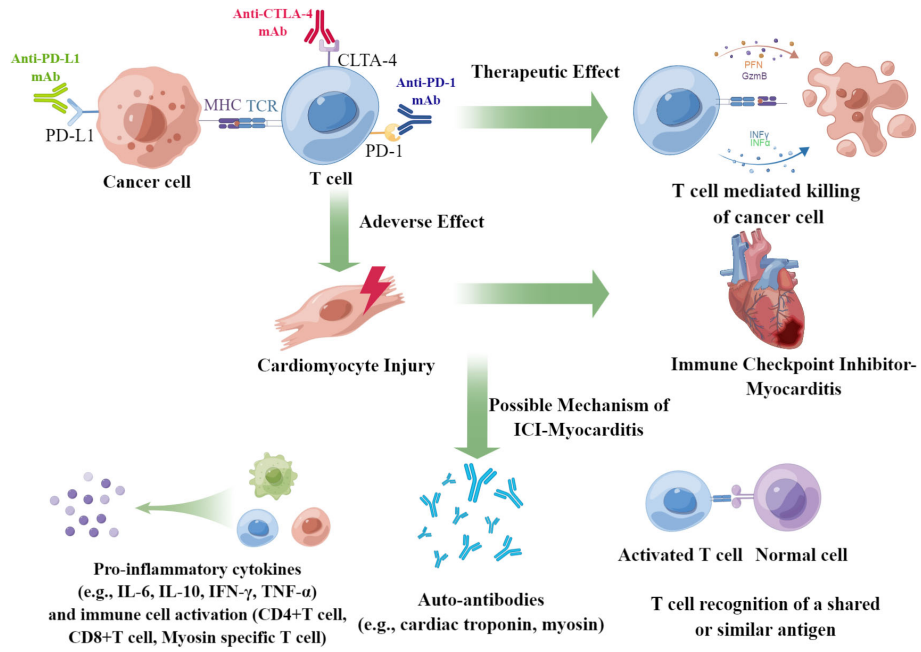

FIGURE 2
Summary of the underlying mechanisms of ICI-associated myocarditis. ICI-associated myocarditis is a serious adverse events of patients of cancers received ICI treatment. The possible mechanism of ICI-associated myocarditis may be due to the elevation of pro-inflammatory cytokines, emergence of auto-antibody, and the T cell recognition of a shared or similar antigen.

of ICI-associated myocarditis reflect an unmet clinical need; therefore, prospective studies should be performed to address this issue. Second, studies are needed to identify predictive markers and medical imaging technologies for patients with high-risk ICI-associated myocarditis, and an endomyocardial biopsy is always required for the final diagnosis. Third, more multicenter clinical trials necessary for formulating and standardizing diagnostic and therapeutic schemes. Further studies should focus on the relative balance between potentially disturbing the cancer treatment and alleviating cardiotoxicities. The most important issue is understanding the pathogenesis of ICI-associated myocarditis at the molecular and cellular levels. Some questions should be addressed: How do ICIs affect immune-cardiac interrelationships? What cardiac antigens are inciting? Why do self-antigens elicit harmful immune responses? Is cell death a critical process in pathogenesis, which cell death patterns are involved if it was true? Does the predominance of arrhythmias primarily reflect disturbances in the conduction system of the heart, or is it generalized by systematic inflammation? Taking into account the greater complexity of human, studies involving the blood and tissues obtained from patients are critical for understanding these mechanisms.

Study limitations. The current investigation also had several limitations, primarily attributable to the retrospective nature of case reports: (1) case reports are inherently subjective, which provide a non-random sample, and often do not allow for causal inferences; (2) although multiple databases were search, publication bias was not entirely ruled out, and mild cases could have been under-reported; particularly, only a few G1 myocarditis cases have been reported; (3) some detailed information on risk factors, diagnostics or management of myocarditis could be missing; (4) patients selected for re-challenge of ICIs were probably those in better clinical condition, and in clinical practice, the decision of re-challenge should be considered carefully on a case-by-case basis; (5) the sample size included in this article was limited and relied on the literature of a small collection of case reports which was not allowed for a more comprehensive quantitative analysis. Furthermore, the associations observed between the patient characteristics and outcomes were not statistically significant, rendering our findings speculative.

Conclusions

ICI-associated myocarditis is an emerging clinical concern that has attracted the attention of cardiologists and oncologists. To integrate information on ICI-associated myocarditis, we recovered and analyzed the largest number of published case reports in our work. A reasonable workflow to manage ICI-associated myocarditis was proposed based on this article as follows: for severe cases (G3 or G4), discontinuation of ICIs, administration of high-dose glucocorticoids (methylprednisolone 1 g/day) and other drugs, plasma exchange, and life support measures; for moderate cases (G1 or G2), discontinuation of ICIs and administration of regular-dose glucocorticoids (methylprednisolone 1-2 mg/kg/day). Once full recovery or improvement is achieved, steroids must be adjusted to low doses (prednisone <10 mg/day) or discontinued. Moreover, re-challenge with ICIs appears feasible in selected patients based on the decisions made by the cardiovascular physician, oncologist, and patient.

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

This study did not require ethical approval.

Author contributions

CW: Data curation, Investigation, Methodology, Writing – original draft. GZ: Data curation, Formal analysis, Investigation, Writing – original draft. ZZ: Data curation, Investigation, Writing – original draft. LY: Data curation, Writing – original draft. SL: Data curation, Writing – original draft. GL: Data curation, Writing – original draft. HW: Data curation, Writing – original draft. JH: writing – original draft. SW: Supervision, Writing – review & editing. NL: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

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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.2023.1275254/full#supplementary-material

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Analysis of vaccine responses after anti-CD20 maintenance in B-cell lymphoma in the Balearic Islands. A single reference center experience

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Introduction: The use of maintenance approaches with anti-CD20 monoclonal antibodies has improved the outcomes of B-cell indolent lymphomas but may lead to significant peripheral B-cell depletion. This depletion can potentially hinder the serological response to neoantigens.

Methods: Our objective was to analyze the effect of anti-CD20 maintenance therapy in a reliable model of response to neoantigens: SARS-CoV-2 vaccine responses and the incidence/severity ofCOVID-19 in a reference hospital.

Results: In our series (n=118), the rate of vaccination failures was 31%. Through ROC curve analysis, we determined a cutoff for SARS-CoV-2 vaccine serologic response at 24 months from the last anti-CD20 dose. The risk of severe COVID-19 was notably higher within the first 24months following the last anti-CD20 dose (52%) compared to after this period (just 18%) (p=0.007). In our survival analysis, neither vaccine response nor hypogammaglobulinemia significantly affected OS. While COVID-19 led to a modest mortality rate of 2.5%, this figure was comparable to the OS reported in the general immunocompetent population. However, most patients with hypogammaglobulinemia received intravenous immunoglobulin therapy and all were vaccinated. In conclusion, anti-CD20 maintenance therapy impairs serological responses to SARS-CoV-2 vaccines.

Discussion: We report for the first time that patients during maintenance therapy and up to 24 months after the last anti-CD20 dose are at a higher risk of vaccine failure and more severe cases of COVID-19. Nevertheless, with close monitoring,

intravenous immunoglobulin supplementation or proper vaccination, the impact on survival due to the lack of serological response in this high-risk population can be mitigated, allowing for the benefits of anti-CD20 maintenance therapy, even in the presence of hypogammaglobulinemia.

KEYWORDS

seroconversion, vaccine failure, B-cell aplasia, SARS/CoV-2, anti-CD20 maintenance

1 Introduction

Anti-CD20 monoclonal antibodies, like rituximab, have enhanced the outcomes of B-cell lymphoma patients when incorporated into many standard chemotherapy regimens (1). However, a significant advancement was achieved with the introduction of maintenance approaches. These involve periodic infusions of anti-CD20 monoclonal antibodies every 2, 3 or 6 months, ensuring continuous anti-CD20 activity against the minimal residual disease that remains after an initial debulking immunochemotherapy. The use of anti-CD20 maintenance approaches has improved the outcome in terms of longer progression-free (PFS) or overall survival (OS) in B-cell lymphomas such as follicular or mantle lymphoma, as shown in PRIMA (2, 3), BRIGHT (4) or LYMA (5) trials. However, other anti-CD20 monoclonal antibodies, such as obinutuzumab, showed better efficacy results, being able to rescue rituximab-resistant patients but at the cost of greater toxicity (6, 7).

Although anti-CD20 maintenance is generally well-tolerated, there is still some significant toxicity mainly related to peripheral B-cell depletion. This B-cell aplasia is generally complete during anti-CD20 maintenance and, after the last dose of anti-CD20, B-cell counts may need several months to recover or even remain prolonged or persistent in some individuals (8). This may impair serological response to neoantigens, including SARS-CoV-2 spike glycoprotein within SARS-CoV-2 vaccines (9) and, although this is well described, to the best of our knowledge no specific study has focused on patients receiving anti-CD20 maintenance approaches increasing the risk of prolonged B-cell aplasia. At the same time, the COVID-19 pandemic offered us the opportunity to study the vaccine response to a particular neoantigen, related to SARS-CoV-2 virus. In this study, we aim to analyze the effect of anti-CD20 maintenance on SARS-CoV-2 vaccine responses and COVID-19 incidence and severity in a single reference hospital.

2 Materials and methods

2.1 Study design

We retrospectively selected from the Pharmacy database of Son Espases University Hospital, those alive patients with B-cell lymphomas treated with anti-CD20 maintenance therapy candidates to be included in the study. Inclusion criteria were having received previous or ongoing frontline anti-CD20 maintenance from January 2003 to August 2022, having received at least one dose of any approved SARS-CoV-2 vaccine by August 2022 and willingness to sign the informed consent. Exclusion criteria included not having received at least one dose of any approved SARS-CoV-2 vaccine by August 2022, anti-CD20 maintenance beyond frontline therapy for B-cell lymphoma, previous administration of anti-SARS/CoV-2 monoclonal antibodies or unwillingness to sign the informed consent. The study was approved by the Balearic Islands ethic committee (L99E19746/2020). Clinical characteristics and outcome were obtained from medical records.

2.2 Humoral immunodeficiency, SARS-CoV-2 vaccination, and COVID-19

Relevant clinical data was retrospectively obtained from electronic medical records of Son Espases University hospital. They included staging and prognostic factors in B-cell lymphoma, humoral immune status assessed by the level of serum immunoglobulins and the need of immunoglobulin supplementation. Hypogammaglobulinemia was defined as IgG levels below normal levels in our center (500 mg/dL). For SARS-CoV-2 serologic assessment we used a high-throughput chemiluminescent immunoassay (CLIA) platform. Vaccination response was evaluated as the rate of seroconversion after vaccine administration. Seroconversion was defined as conversion from negative to protective titers of IgG anti-S (>260 AU/mL). Vaccination failure was defined as not achieving protective titers after at least 1 vaccination dose. COVID-19 severity was analyzed using Radiographic Assessment of Lung Edema (RALE) score (10, 11) in those patients available (severe and those requiring ICU admission), as they offers an objective, rapid and widely available tool that can be extremely useful, especially when integrated with other clinical data. However, from a practical point of view COVID-19 severity was classified as asymptomatic, mild, severe, or requiring ICU admission.

2.3 Statistical methods

Variables following binomial distributions (i.e.: response rate), were expressed as frequencies and percentages. Comparisons

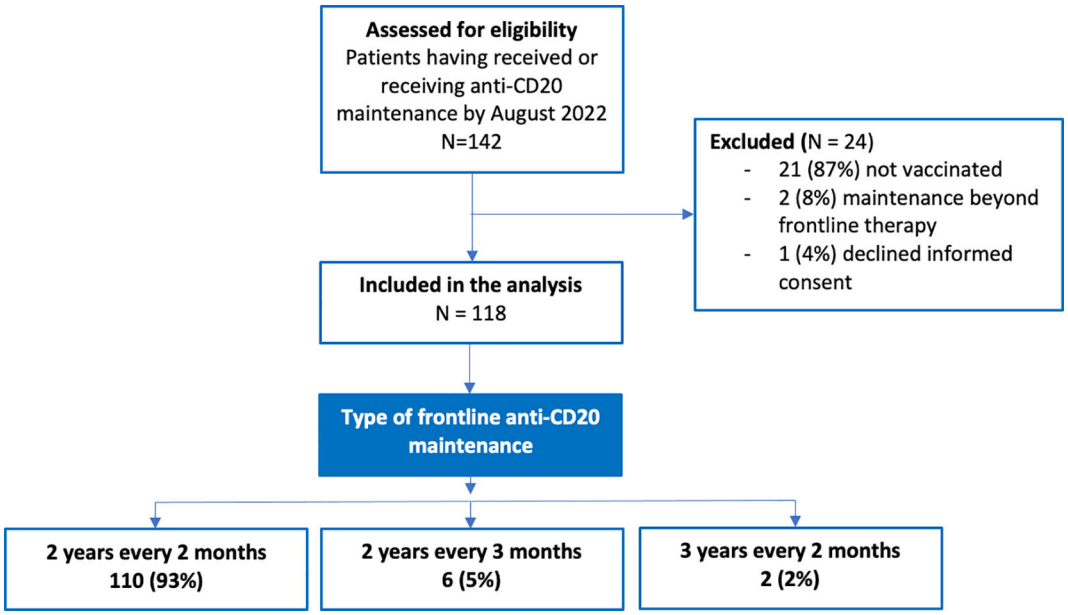

FIGURE 1
Flow-chart of the patients included and excluded from the study.

between qualitative variables were done using the Fisher Exact Test or Chi-square. Comparisons between quantitative and qualitative variables were performed through non-parametric tests (U of Mann-Whitney or Kruskal-Wallis). To analyze the moment of recovery of the serological response to SARS-CoV-2 vaccination after the last dose of anti-CD20 maintenance, ROC curves were used. Time to event variables (OS and PFS) were measured from the date of therapy onset and were estimated according to the Kaplan-Meier method. Comparisons between the variables of interest were performed by the log-rank test. All p-values reported were 2-sided, and statistical significance was defined at $p < 0{\cdot}05$.

3 Results

3.1 Characteristics of the patients

From the Pharmacy database of our institution, we identified 142 patients who received anti-CD20 maintenance from July-2003 to May-2022. 118 patients fulfilled inclusion criteria and signed the informed consent. In Figure 1 we depict a flow-chart detailing the patients included in the study. Of note, 24 patients were excluded for the following reasons: 21 (87%) had not been vaccinated by August 2022, 2 (8%) had not received frontline anti-CD20 maintenance for B-cell lymphoma and 1 (4%) declined to sign the informed consent. Main characteristics of patients are showed in Table 1. Briefly, median age was 64 years (22–89), 52% of cases were male, the most frequent diagnosis was follicular lymphoma (51%), followed by diffuse large B-cell lymphoma (17%) and mantle lymphoma (10%), most cases with advanced III-IV AA stage (82%) and 26% with B-symptoms.

Regarding previous therapy, most patients received R-bendamustine (46%) or R-CHOP/R-CVP (29%) as induction regimen. Median number of induction cycles was 6 (1-9). Most patients received the anti-CD20 maintenance therapy in the frontline setting and using a 2-years every 2-months approach (93%). Median maintenance cycles were 12 (1-18). Associated to anti-CD20 maintenance, 51 patients (43%) showed secondary hypogammaglobulinemia.

3.2 SARS-CoV-2 vaccine response and severity of COVID-19

As shown in Table 2, median SARS-CoV-2 vaccine doses received were 3 (1-4), most patients having 3 or 4 doses (83%). Median time since last anti-CD20 dose was 48 months (0-189). Median IgG anti-S quantitative anti-SARS-CoV-2 title was 893.2 AU/mL (0->40000). The rate of vaccination failure of our series was 31%. Median time since last dose of anti-CD20 of patients with vaccination failures was significantly lower (2 months) compared with patients with vaccination success (78 months) ($p<0.001$). Using ROC curves, we obtained a cutoff for SARS-CoV-2 vaccine serologic response at 24 months from last anti-CD20 dose (area under curve of 0.83; $p<0.001$) (Figure 2). From this cutoff, 90% of patients obtained a successful IgG anti-S level compared to just 36% below that cutoff ($p<0.001$), which represent a vaccination failure of 63.8%, with no differences between patients vaccinated during anti-

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TABLE 1 Main characteristics of patients.

Median age (range)	64 (22-89)
Sex (Male/Female) (%)	61 (52%)/57 (48%)
Diagnosis (%): - Follicular lymphoma - DLBCL - Mantle lymphoma - Marginal lymphoma - CLL/SLL - Other	 60 (51%) 20 (17%) 12 (10%) 11 (9%) 7 (6%) 8 (7%)
Ann Arbor Stage (%): - I-II - III-IV	 21 (18%) 97 (82%)
B-symptoms (%):	31 (26%)
Induction therapy (%): - R-bendamustine - R-CHOP/R-CVP - R monotherapy - R-GemOx - Fludarabine- based - Intensive approaches - Other	 54 (46%) 34 (29%) 14 (12%) 9 (8%) 3 (2%) 3 (2%) 1 (1%)
Median induction cycles (range)	6 (1-9)
Anti-CD20 maintenance (%): - 2 years every 2 months - 2 years every 3 months - 3 years every 2 months	 110 (93%) 6 (5%) 2 (2%)
Median maintenance cycles (range)	12 (1-18)
Secondary hypogammaglobulinemia (%): - Yes - No	 51 (43%) 67 (57%)
Intravenous immunoglobulin administration (%): - Yes - No	 30 (25%) 88 (75%)

DLBCL, diffuse large B-cell lymphoma; CLL/SLL, chronic lymphoid leukemia/small lymphocytic lymphoma; R-CHOP, rituximab, cyclophosphamide, doxorrubicin, prednisone; R-CVP, rituximab, cyclophosphamide, prednisone; R-GemOx, rituximab, gemcitabine, oxaliplatin.

CD20 maintenance (63.6%) or during the first 24 months after the last anti-CD20 dose (63.9%). Similarly, there were no differences in the rate of vaccination failure between patients receiving 1-2 vaccine doses and those receiving 3-4 (p=0.6).

Considering severity of COVID-19 infection in this series of B-cell lymphomas treated with anti-CD20 maintenance, 63 cases had a COVID-19 infection (53%). From these patients, 33% suffered severe or requiring intensive care COVID-19 while in 67% was mild or asymptomatic. More importantly, the risk of severe COVID-19 was much higher during the first 24 months after last anti-CD20 dose (52%) than after this cutoff (just 18%) (p=0.007). Table 2 shows main SARS-CoV-2 and COVID-19 characteristics of the series.

3.3 Impact of vaccine response, hypogammaglobulinemia and COVID-19 infection on survival

Median follow-up of our series from frontline therapy was 85 months (95%CI: 70-100). 7y-OS was 96% (95%CI: 92-100).

TABLE 2 SARS-CoV-2 and COVID-19 infection data.

Median vaccine doses (range)	3 (1-4)	p
Vaccine doses: - 1 - 2 - 3 - 4	 2 (2%) 17 (14%) 82 (69%) 17 (14%)	N/A
Median months since last anti-CD20 dose (range)	48 (0-189)	N/A
Median IgG anti-S quantitative anti-SARS-CoV-2 (AU/mL)	893.2 (0->40000)	N/A
Vaccination failure (<260 AU/mL)	37 (31%)	N/A
COVID-19 infection (%)	63 (53%)	N/A
Severity of COVID-19 infection: - Asymptomatic - Mild - Severe - Requiring intensive care	 10 (16%) 32 (51%) 15 (24%) 6 (9%)	N/A
Vaccination response according to time from end of anti-CD20 maintenance: - 0-24 months - >24 months	 17/47 (36%) 64/71 (90%)	<0.001
Risk of severe COVID-19 according to time from end of anti-CD20 maintenance: - 0-24 months - >24 months	 15/29 (52%) 6/34 (18%)	0.007

Univariate analysis of clinical factors associated with OS is shown in Table 3; Figure 3. There was no significant impact of vaccine response (p=0.29) or hypogammaglobulinemia (p=0.78). However, the incidence of COVID-19 was associated with a significantly lower OS (p=0.025). Although the difference was only 8% (100% *vs* 92% with COVID19), this difference was related to severe cases (2/15) or ICU cases (1/6), in which the mortality rate associated with COVID-19 was 13% and 17%, respectively. Overall, causes of death were COVID-19 in 3 patients (2.5%) and stroke in 2 cases (1.7%).

In this series, despite potential B-cell immunosuppression and high rates of B-cell ablation, the COVID-19-related mortality rate was 2.5% in 118 cases. Although this is significant, it appears modest. However, it is important to note that all patients were vaccinated, and a majority of those with hypogammaglobulinemia received intravenous immunoglobulin therapy (59%).

When we evaluated other immunosuppressive factors such as the type of induction therapy, we observed a significantly higher incidence of COVID-19 among patients administered induction therapy with BR at 65%, compared to those treated with R-CHOP/R-CVP at 35% (p=0.036). However, we should note that BR has been the preferred therapy for the most recent patients. Consequently, the median time from the last anti-CD20 dose was 37 months for BR patients and 82 months for R-CHOP/R-CVP patients (p=0.004). Importantly, this higher incidence of COVID-19 did not translate into a significant difference in 7-year overall survival (7y-OS) (93% *vs*. 97%; p=0.36) or in COVID-19-specific death rates (3.7% *vs*. 2.9%; p=0.74).

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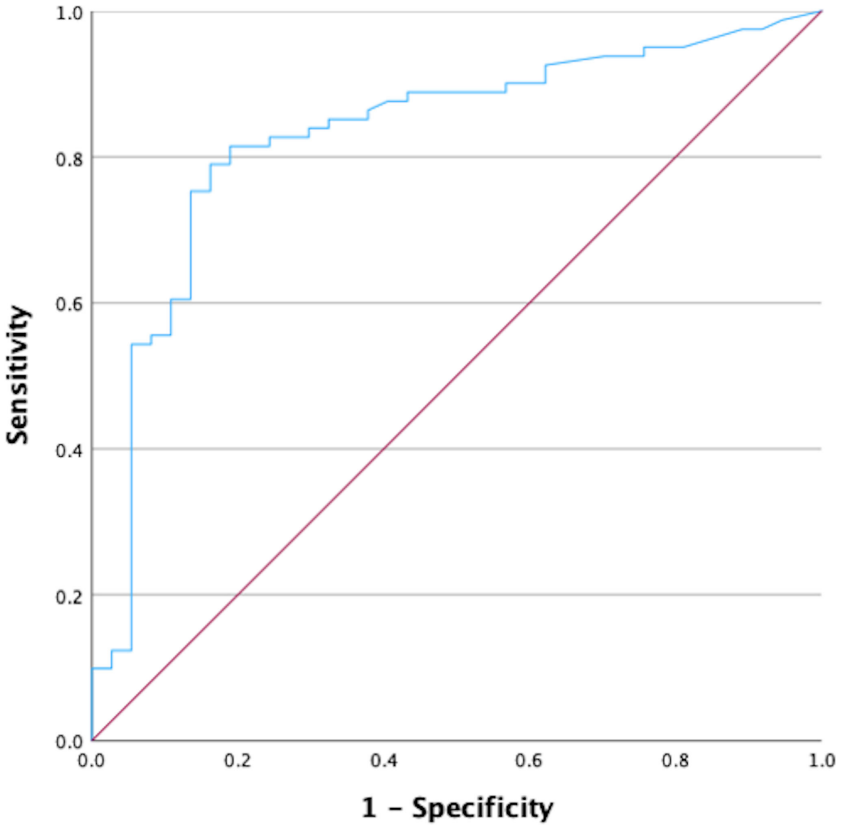

FIGURE 2
ROC curve to identify the moment of restoration of serological responses to SARS-CoV-2 vaccines after anti-CD20 maintenance.

TABLE 3 Univariate analysis of clinical factors on overall survival.

	7y-OS (95%CI)	p
Age: - 18-60 - >60	 100% (NA) 93% (86-100)	0.048
Sex: - Male - Female	 92% (84-99) 100% (NA)	0.019
AA stage: - I-II - III-IV	 100% (NA) 95% (90-100)	0.32
B-symptoms: - Yes - No	 97% (90-100) 96% (91-100)	0.78
Diagnosis: - Follicular lymphoma - Non-follicular indolent - Mantle-cell lymphoma - DLBCL	 94% (88-100) 100% (NA) 100% (NA) 93% (79-100)	0.64
Induction therapy: - Benda-based - CHOP-like - Rituximab monotherapy - Other	 93% (86-100) 97% (90-100) 100% (NA) 100% (NA)	0.36
Seroconversion after vaccine: - Success - Failure	 97% (94-100) 90% (78-100)	0.29
Hypogammaglobulinemia: - Yes - No	 98% (93-100) 94% (88-100)	0.78
Intravenous immunoglobulins (if hypogammaglobulinemia): - Yes - No	 100% (NA) 95% (85-100)	0.92
COVID19: - Yes - No	 92% (85-100) 100% (NA)	0.025
Severity of COVID-19 infection: - Asymptomatic - Mild - Severe - Requiring intensive care	 100% (NA) 100% (NA) 71% (43-100) 80% (45-100)	0.032

NA, not available.

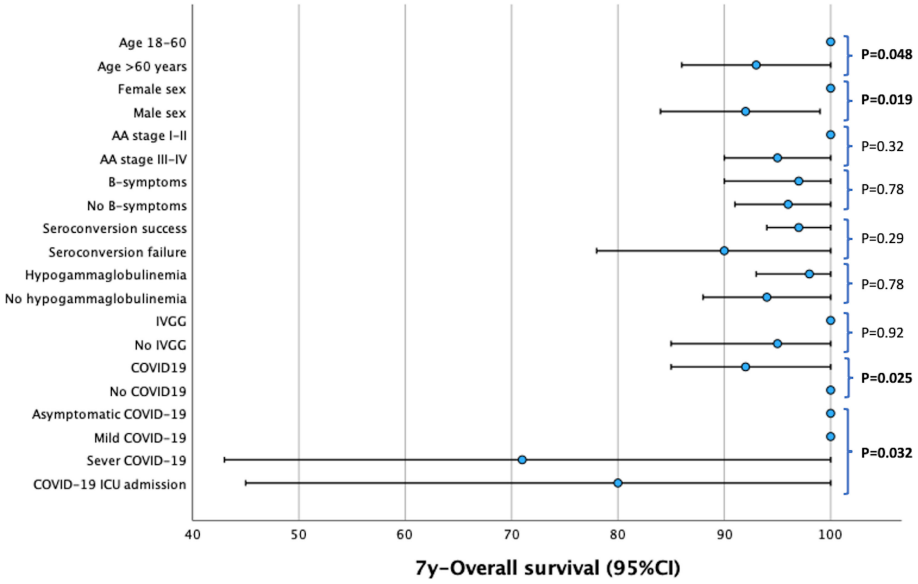

FIGURE 3
Forest-plot of the univariate analysis of overall survival.

4 Discussion

We present the first data set about the impact on SARS-CoV-2 vaccines efficacy of a particular approach in anti-CD20 therapy for B-cell malignancies, the maintenance treatment. Most anti-CD20 maintenance approaches imply a severe long-term (2-3 years) B-cell ablation. This fact has generated important concerns about their safety in the context of the SARS-CoV-2 pandemic to both physicians and patients. Such concerns may lead to preclude the use of these anti-CD20 maintenance, which consequently may imply a worse control of the B-cell malignancy.

Anti-CD20 therapy efficiently depletes peripheral B-cells that represent only 2% of the total B-cell population. Similarly, there is an impact on peripheral lymphoid tissues but lower on long-lived plasma cells, which do not express the anti-CD20 antigen (12–15). After short-term anti-CD20 induction schemes, such as R-CHOP-like regimens or rituximab monotherapy, the peripheral blood B-cell compartment has been described to recover within 6-9 months after the last anti-CD20 dose (8, 12). However, there is less information regarding long-term anti-CD20 approaches such as anti-CD20 maintenance, but this data could be obtained evaluating a surrogate biomarker of proper B-cell function such as seroconversion after vaccination. To identify the point in which there is a significant change in the ability of seroconvert after vaccination, we evaluated only patients who receiving, or had previously received, anti-CD20 maintenance as part of their frontline therapy. We excluded those who received anti-CD20 maintenance in the second or subsequent treatment lines (Figure 1).

In our study, to our knowledge also for the first time, we used ROC curves to calculate the length of main impairment of seroconversion after vaccination in anti-CD20 maintenance approaches: 24 months since the last anti-CD20 dose. The median rate of vaccination failure was 31% in our series, being even higher (64%) during maintenance and up to the first 24 months after the last dose of anti-CD20 maintenance. Beyond this moment, the seroconversion rate improved until 90% (10% vaccination failure rate), showing a much longer impairment on B-cell function after anti-CD20 maintenance therapy compared to short induction regimens.

A recent metanalysis in patients mostly receiving short courses of anti-CD20 therapy, reported even lower seroconversion rates for 2 doses of the pandemic influenza vaccine in patients on active anti-CD20 therapy (12%) and that apparently improved with the time since the last anti-CD20 dose. When comparing patients on active anti-CD20 therapy with controls, the differences in seroconversion rates were less pronounced by an average of 6 to 12 months from last anti-CD20 dose and were similar beyond 12 months (16). To overcome this prologued time to seroconversion it has been proposed to delay anti-CD20 therapy until after vaccination (17). Another recent study proposed that the optimal interval for SARS-CoV-2 vaccination after the final dose of anti-CD20 is 5.5 months, but mostly in patients receiving short courses of anti-CD20 therapy (18).

In the context of our study on SARS-CoV-2 serologic assessment, the high sensitivity and specificity of the CLIA method is especially crucial, ensuring that the antibody responses of individuals, even if weak, are accurately captured. This ensures the validity and robustness of our findings, particularly when drawing conclusions about the impact of treatments or interventions on antibody production and response (19).

The other interesting contribution that we can extract form our series is that anti-CD20 maintenance approaches are safe even in patients with a high degree of humoral immunodeficiency during especially risky situations such as the recent SARS-CoV-2 pandemic. In our patients we had no significant impact on survival of seroconversion failure after SARS-CoV-2 vaccination

or hypogammaglobulinemia. However, 25% of these patients received intravenous immunoglobulins therapy, mainly those having symptomatic hypogammaglobulinemia (59%). Patients receiving anti-CD20 maintenance approaches have an increased risk of hypogammaglobulinemia, some of them associated with recurrent infections (20). Some guidelines recommend administering intravenous immunoglobulins to patients with 2 or more non-neutropenic infections in a 6-month period of time (21) and this is also our standard approach. Furthermore, in our series all patients were vaccinated. It is well described that even patients who do not respond to the SARS-CoV-2 vaccines, may develop some degree of T-cell sensibilization that could in part protect or reduce the severity of COVID19 (22).

Another aspect warranting discussion involves the controversial impact of induction immunochemotherapy based on bendamustine (BR) compared to alternatives such as R-CHOP/R-CVP or other options. In our study, we noted a higher incidence of COVID-19 among patients treated with BR, potentially attributable to a higher immunosuppressive activity of bendamustine but also to its status as the preferred therapy for the most recent indolent lymphoma cases, and the corresponding shorter interval since the last anti-CD20 dose. However, it is pivotal to highlight, as previously mentioned, that this increased incidence of COVID-19 did not correlate with a higher incidence of severe COVID-19, shorter OS or higher COVID-19-specific death rates.

Like all retrospective studies, our work is subject to potential bias. Furthermore, we included only those patients who were alive in May 2022, a time when less aggressive SARS-CoV-2 variants were in circulation. Although the impact of these variants might be partially compensated by the less stringent lockdown measures in place, they could have influenced the OS analysis. However, these factors would not affect the seroconversion rates.

Although in our series, vaccination failure or hypogammaglobulinemia did not impact outcomes, COVID19 still had a small but significant effect on mortality (2.5%) and OS. While this percentage is low when looking at the entire series, it is higher for cases that were severe or required ICU admission (13% and 17%, respectively). However, these figures do not significantly differ from the rates reported for the general immunocompetent population (23, 24). They still represent acceptable mortality rates considering the potential B-cell immunosuppression in this group of patients. Given this, we can hypothesize that with the above-mentioned prophylactic measures and close monitoring, there is no justification to broadly preclude the use of anti-CD20 maintenance to any of the well-demonstrated clinical settings in which these approaches have shown important benefit in terms of progression-free survival or even lymphoma cure. Additionally, during the COVID-19 pandemic several anti-SARS-CoV-2 monoclonal antibodies, such as cilgavimab/tixagevimab (25) or sotrovimab (26) have been developed that could help to compensate anti-CD20 maintenance-associated humoral immunodeficiency.

We conclude that anti-CD20 maintenance therapy impairs serological responses to SARS-CoV-2 vaccines. To our knowledge we report for the first time that patients during maintenance and up to 24 months after finishing the last anti-CD20 dose are at a higher-risk of vaccine failure and more severe cases of COVID-19. However, a close monitoring, intravenous immunoglobulin supplementation, if necessary, proper vaccination if available or the use of specific monoclonal antibodies in the case of COVID-19 infection, may overcome the impact on survival of this lack of serological response in high-risk population. In other words, with these measures, anti-CD20 maintenance is a safe procedure that should not be avoided or discontinued even in the case of hypogammaglobulinemia.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Balearic Islands ethic committee. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

AG: Conceptualization, Formal Analysis, Investigation, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing. AA: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. MG-R: Conceptualization, Formal Analysis, Investigation, Validation, Writing – original draft, Writing – review & editing. SP: Data curation, Resources, Validation, Writing – review & editing. LG-M: Data curation, Resources, Validation, Writing – review & editing. JM-S: Data curation, Investigation, Resources, Validation, Writing – review & editing. TR: Data curation, Investigation, Resources, Validation, Writing – review & editing. MG-G: Data curation, Investigation, Methodology, Supervision, Validation, Writing – review & editing. JF: Conceptualization, Data curation, Methodology, Supervision, Writing – review & editing. OV: Data curation, Methodology, Supervision, Validation, Writing – review & editing. RA: Data curation, Methodology, Validation, Writing – review & editing. ASl: Conceptualization, Methodology, Supervision, Validation, Writing – review & editing. ASm: Data curation, Resources, Validation, Writing – review & editing. LB: Data curation, Resources, Validation, Writing – review & editing.

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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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The association between aspirin use and immune-related adverse events in specific cancer patients receiving ICIs therapy: analysis of the FAERS database

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Background: The promise of immune checkpoint inhibitors (ICIs) therapy in cancer treatment is tempered by the occurrence of immune-related adverse events (irAEs). Many patients undergoing ICIs also take aspirin, but the association between aspirin and irAEs is not well understood.

Methods: This study analyzed adverse reaction data associated with the use of ICIs in the US Food and Drug Administration (FDA) Adverse Event Reporting System FDA Adverse Event Reporting System database, from the approval date of each drug until 1 October 2022. Multivariate logistic regression was employed to assess the association of aspirin use with irAEs in patients receiving ICIs.

Results: The results indicated that aspirin use was associated with an increased risk of irAEs in a pan-cancer analysis, with a more pronounced association in specific cancer types such as lung cancer, mesothelioma, and pancreatic cancer. However, in lymphoma, aspirin use was associated with a reduced risk of irAEs. Furthermore, aspirin use was associated with an increased risk of certain irAEs, such as anemia, colitis, myocarditis, myositis, pancreatitis, pericarditis, and pneumonia, while it was associated with a reduced risk of rash, Stevens-Johnson syndrome, and thyroiditis.

Conclusion: This study has unveiled an association between aspirin use and irAEs in cancer patients receiving ICIs therapy, emphasizing the need for individualized consideration of patients' medication history when devising cancer treatment plans to enhance efficacy and reduce risks.

KEYWORDS

immune checkpoint Inhibitors (ICIs), aspirin, immune-related adverse events (irAEs), cancer, US food and drug administration adverse event reporting system (FAERS) database

1 Introduction

ICIs therapy is a groundbreaking approach to treating tumors that leverages the immune system to combat malignancies. This approach enhances immune-mediated tumor clearance by blocking negative signals between cancer cells and immune cells (Waldman et al., 2020; Morad et al., 2021). To this end, ICIs that target programmed cell death-1 (PD-1)/programmed death ligand-1 (PD-L1) as well as cytotoxic T lymphocyte-associated protein 4 (CTLA-4) have been developed and employed by researchers in clinical practice (Waldman et al., 2020; Morad et al., 2021). As mounting evidence supports their efficacy and synergistic effects with other cancer treatments, ICIs are increasingly being utilized as a key component in the treatment of many types of cancer, such as melanoma, lung cancer and esophageal cancer (Gadgeel et al., 2020; Rudin et al., 2020; Doki et al., 2022; Livingstone et al., 2022). However, it is important to note that, to date, ICIs remains ineffective for several cancer types, for instance, pancreatic cancer (Bockorny et al., 2022). In some cases, ICIs have not yet attained the status of standard care, as seen in breast cancer (Debien et al., 2023).

However, a notable issue arising from the increasing use of ICIs in clinical practice is their uncontrolled additive impact on the immune system, resulting in irAEs. ICIs manifest unique patterns of toxicity distinct from conventional chemotherapy or other biological agents, often stemming from hyperactive immune reactions against normal organs. irAEs can affect any organ system, including the skin, gastrointestinal tract, cardiovascular system, and endocrine system, among others (Brahmer et al., 2018; Schneider et al., 2021). The frequency of irAEs ranged from 66.4% to 86.8% for all grades, and from 14.1% to 28.6% for grade 3 or higher (Xu et al., 2018). irAEs may be influenced by the patient's genetic background and microbiome, as well as by treatment-related factors such as combination medication (Jelinic et al., 2018; Cortellini et al., 2020). The mechanism of irAEs is not fully understood but may be related to the overactivation of innate and adaptive immunity caused by the disruption of immune balance by immunotherapy (Pauken et al., 2019). Since the occurrence of irAEs restricts the use of ICIs, it is necessary to further understand the mechanism and influencing factors of irAEs.

Drug-drug interactions (DDI) are a significant focus in the field of systemic anti-cancer treatment. Previous studies have found that combination therapy has an important impact on the outcome of immunotherapy and irAEs. For example, the use of antibiotics and proton pump inhibitors (PPIs) has been associated with poorer outcomes in patients with ICIs (Kostine et al., 2021). Aspirin has become widely used in modern medicine, primarily due to its ability to inhibit the cyclooxygenase (COX) pathway and effectively treat inflammation, pain, and various cardiovascular diseases (Fijałkowski et al., 2022). In recent years, aspirin has also been found to have a well-documented role in the prevention and treatment of tumors (Algra and Rothwell, 2012; Rothwell et al., 2012), especially in colorectal cancer (Rothwell et al., 2010; Drew and Chan, 2021). With the innovation in the field of cancer treatment and the emergence of a new therapy, namely, immunotherapy, researchers have gradually paid attention to the relationship between aspirin and immunotherapy. Recent clinical studies have suggested that the combination of aspirin and ICIs is associated with better outcomes (Cortellini et al., 2020; Zhang et al., 2021). In addition, Aspirin use and its relationship to irAEs were rarely addressed in these studies. Given the widespread acceptance of ICIs into standard practice, it is crucial to gain a better understanding of the association between aspirin treatment and irAEs.

FAERS is a comprehensive drug adverse reaction database maintained by the FDA. Its advantages include broad coverage of adverse events from clinical trials to market use, timely updates, comprehensive drug information, large-scale data for analysis, and reliable reporting from healthcare professionals and consumers. It is a trusted resource for monitoring and reporting drug adverse reactions, and helps to inform better clinical practice and healthcare decision-making. To date, no systematic evaluation of the association of aspirin with irAEs has been published. Therefore, our aim was to determine the association between aspirin use and irAEs in patients receiving immunotherapy by analyzing the data in FAERS. Our research affirms that aspirin users exhibited a higher risk of irAEs when compared to non-aspirin users. Nonetheless, this association displayed variability across distinct cancer types, adverse events, and ICIs.

2 Methods

2.1 Data sources

The study utilized data from the FAERS database, a public repository that houses information on adverse events and medication errors reported to the FDA. This database is an essential tool for the FDA's post-marketing safety surveillance program for drug and therapeutic biologic products. All data used for this analysis can be accessed at https://fis.fda.gov/sense/app/95239e26-e0be-42d9-a960-9a5f7f1c25ee/sheet/7a47a261-d58b-4203-a8aa-6d3021737452/state/analysis.

2.2 Data collection and screening

Adverse event (AE) reports from ICIs in the FAERS database were collected for this retrospective study. The analysis included every report from the date of each drug's FDA approval until 1 October 2022. ICIs mainly consists of PD-1 inhibitors (Nivolumab, Pembrolizumab, Cemiplimab, Sintilimab, Camrelizumab, Tislelizumab, Toripalimab), PD-L1 inhibitors (Durvalumab, Atezolizumab, Avelumab), CTLA-4 inhibitors (Ipilimumab, Tremelimumab, Quavonlimab, Bms-986249), Lymphocyte-activation gene 3 (LAG-3) inhibitors (Opdualag, Relatlimab, Favezelimab, Fianlimab), PD-1/LAG-3 bispecific inhibitors (Nivolumab\Relatlimab-Rmbw, Tebotelimab). According to the patient's medication, the treatment regimen were classified as monotherapy, dual immunotherapy, immunotherapy combined with targeted therapy, immunotherapy combined with chemotherapy, and immune combined antibody drug conjugates (ADC). We defined the use of aspirin during immunotherapy as aspirin users. irAEs were defined using AE terminology from the peer-reviewed immune-related adverse event (irAE) management guidelines (Martins et al., 2019). Patients with at least one irAE were categorized into the irAE group. The irAEs were sorted into primary system organ classes according to the Medical Dictionary for Regulatory Activities (Jing et al., 2022).

TABLE 1 Baseline feature.

Characteristics		With ASA	Without ASA	*p*-Value
		n = 5,359	n = 117,745	
Sex	Female	1,424 (26.6%)	41,676 (35.4%)	$p < 0.001$
	Male	3,909 (72.9%)	70,432 (59.8%)	
	Not specified	26 (0.5%)	5,637 (4.8%)	
age		68.9 (9.0)	63.9 (12.5)	$p < 0.001$
ICIs type	PD-1 inhibitor	2,847 (53.1%)	67,808 (57.6%)	$p < 0.001$
	PD-L1 inhibitor	1,193 (22.3%)	20,122 (17.1%)	
	CTLA-4 inhibitor	1,297 (24.2%)	29,356 (24.9%)	
	LAG-3 inhibitor	19 (0.6%)	239 (0.2%)	
	PD-1/LAG-3 inhibitor	3 (0.1%)	220 (0.2%)	
Cancer type	Bile duct cancer	15 (0.3%)	493 (0.4%)	$p < 0.001$
	Brain cancer	17 (0.3%)	641 (0.5%)	
	Breast cancer	57 (1.1%)	2,356 (2.0%)	
	Cervical cancer	8 (0.1%)	488 (0.4%)	
	Colorectal cancer	98 (1.8%)	1870 (1.6%)	
	Endometrial cancer	38 (0.7%)	1,227 (1.0%)	
	Esophageal cancer	48 (0.9%)	1,382 (1.2%)	
	Gastric cancer	52 (1.0%)	2,706 (2.3%)	
	Head and neck cancer	119 (2.2%)	2,996 (2.5%)	
	Liver cancer	159 (3.0%)	3,589 (3.0%)	
	Lung cancer	1,532 (28.6%)	29,212 (24.8%)	
	Lymphoma	81 (1.5%)	1732 (1.5%)	
	Melanoma	1,095 (20.4%)	25,904 (22.0%)	
	Mesothelioma	70 (1.3%)	1,256 (1.1%)	
	Metastatic tumor	112 (2.1%)	2,303 (2.0%)	
	Neuroendocrine tumor	13 (0.2%)	339 (0.3%)	
	Ovarian cancer	49 (0.9%)	1,212 (1.0%)	
	Pancreatic cancer	243 (4.5%)	1,691 (1.4%)	
	Prostate cancer	140 (2.6%)	1,025 (0.9%)	
	Renal cancer	591 (11.0%)	12,087 (10.3%)	
	Sarcoma	55 (1.0%)	843 (0.7%)	
	Skin cancer	39 (0.7%)	662 (0.6%)	
	Thyroid cancer	9 (0.2%)	299 (0.3%)	
	Urothelial tract cancer	165 (3.1%)	3,063 (2.6%)	
	Other cancers	554 (10.3%)	18,369 (15.6%)	
irAEs	Yes	1,294 (24.1%)	25,205 (21.4%)	$p < 0.001$
	No	4,065 (75.9%)	92,540 (78.6%)	

ASA: Aspirin.

Cancer types	AE/Total (With ASA)	AE/Total (Without ASA)	OR(95%CI)	FDR p
Pan-cancer	1294/5359	25205/117745	1.18(1.10-1.26)	<0.001
Bile duct	6/15	79/493	4.18(1.25-14.04)	0.100
Brain	1/17	63/641	0.59(0.08-4.66)	0.998
Breast	16/57	444/2356	1.48(0.80-2.74)	0.671
Cervical	2/8	109/488	1.20(0.22-6.53)	0.998
Colorectal	19/98	307/1870	1.21(0.71-2.05)	0.998
Endometrial	7/38	235/1227	0.89(0.38-2.09)	0.998
Esophageal	9/48	197/1382	1.10(0.48-2.56)	0.998
Gastric	3/52	378/2706	0.37(0.11-1.21)	0.378
Head and neck	14/119	388/2996	0.92(0.52-1.64)	0.998
Liver	19/159	459/3589	0.97(0.58-1.61)	0.998
Lung	401/1532	6440/29212	1.24(1.10-1.40)	0.003
Lymphoma	5/81	382/1732	0.27(0.11-0.67)	0.029
Melanoma	341/1095	7790/25904	1.14(1.00-1.31)	0.221
Mesothelioma	28/70	240/1256	2.90(1.75-4.82)	<0.001
Metastatic	28/112	550/2303	1.04(0.66-1.64)	0.998
Neuroendocrine	1/13	57/339	0.45(0.06-3.71)	0.998
Ovarian	10/49	213/1212	1.45(0.67-3.14)	0.913
Pancreatic	68/243	228/1691	2.51(1.79-3.51)	<0.001
Prostate	24/140	206/1025	0.86(0.53-1.39)	0.998
Renal	133/591	2647/12087	1.07(0.88-1.31)	0.998
Sarcoma	6/55	117/843	0.82(0.33-2.04)	0.998
Skin	6/39	99/662	1.14(0.45-2.85)	0.998
Thyroid	3/9	58/299	2.54(0.51-12.77)	0.728
Urothelial tract	31/165	516/3063	1.11(0.74-1.67)	0.998

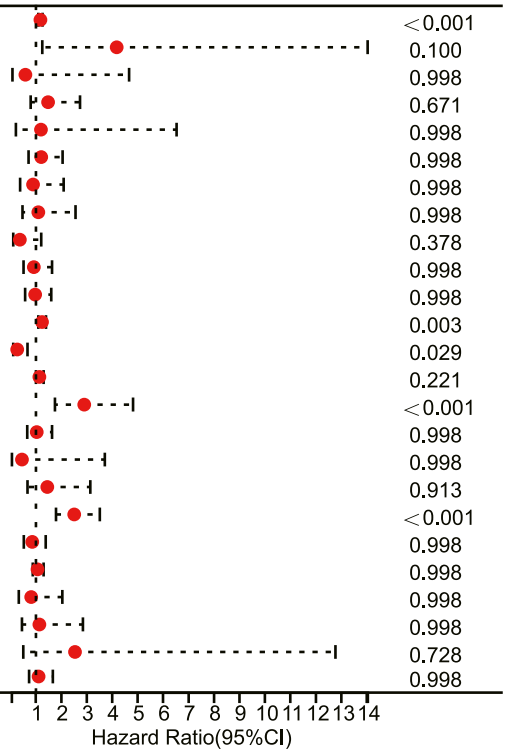

FIGURE 1
The forest plot showing the association between aspirin and irAEs in different cancer types among patients receiving immunotherapy. ASA: Aspirin.

2.3 Statistical analysis

In this study, multivariable logistic regression was utilized to analyze adjusted odds ratios (OR) for evaluating the association between aspirin use and irAEs. The model included covariates such as age, sex, ICIs drugs, and treatment regimen. To account for multiple comparisons, Benjamini–Hochberg adjustment was performed using the "p.adjust" function in the "stats" R package. All comparisons are two tailed, and statistical significance was set at an FDR adjusted $p < 0.05$. The data were processed and analyzed using R statistical software version 4.2.1. On the overall population, we conducted multivariate regression analyses grouping by different tumor types, types of adverse reactions, and system organ classes (SOCs) to determine the impact of aspirin use on irAEs in patients treated with ICIs. Additionally, to further determine if different ICIs had an effect on the results, we performed multivariate logistic regression analyses in patients treated with PD-1 inhibitors, PD-L1 inhibitors, and CTLA-4 inhibitors, respectively.

3 Results

3.1 Baseline characteristics of patients

We collected information on 123,104 patients from FAERS and conducted a multivariate regression analysis (Table 1). Out of these patients, 70,655 were treated with PD-1 inhibitors, 21,315 were treated with PD-L1 inhibitors, 30,653 were treated with CTLA-4 inhibitors, 258 were treated with LAG-3 inhibitors, and 223 were treated with PD-1/LAG-3 bispecific inhibitors (Table 1). Moreover, 5,359 patients (4.4%) reported also taking aspirin (Table 1).

3.2 Association of aspirin treatment with irAEs in different cancer types

The multivariate logistic regression analysis results revealed that aspirin use was associated with an increased risk of irAEs in the pan-cancer analysis (odds ratio (OR) 1.18, 95% confidence interval (CI) 1.10–1.26, FDR adjusted $p < 0.001$) (Figure 1). After excluding cancer types with a sample size of less than 200, we included 24 cancer types for analysis (Table 1). The further analysis indicated that aspirin use was linked to a higher risk of irAEs in specific cancer types. Specifically, aspirin use was significantly associated with an increased risk of irAEs in lung cancer (OR 1.24, 95% CI 1.10–1.40, FDR adjusted $p = 0.003$) (Figure 1), mesothelioma (OR 2.90, 95% CI 1.75–4.82, FDR adjusted $p < 0.001$) (Figure 1), and pancreatic cancer (OR 2.51, 95% CI 1.79–3.51, FDR adjusted $p < 0.001$) (Figure 1). In contrast, aspirin use was linked to a lower risk of irAEs in lymphoma (OR 0.27, 95% CI 0.11–0.67, FDR adjusted $p = 0.029$) (Figure 1).

irAEs	AE/Total (With ASA)	AE/Total (Without ASA)	OR(95%CI)	FDR p
anaemia	163/5359	2683/117745	1.24(1.06-1.47)	0.042
arthralgia	89/5359	1737/117745	1.26(1.01-1.56)	0.119
arthritis	25/5359	682/117745	0.86(0.58-1.29)	0.889
colitis	234/5359	3890/117745	1.46(1.27-1.68)	<0.001
conjunctivitis	8/5359	170/117745	1.15(0.56-2.34)	1.000
dermatomyositis	2/5359	96/117745	0.43(0.11-1.75)	0.633
encephalitis	33/5359	509/117745	1.48(1.03-2.11)	0.114
enteritis	24/5359	314/117745	1.66(1.08-2.56)	0.083
gastritis	15/5359	383/117745	1.01(0.60-1.70)	1.000
glomerulonephritis	2/5359	63/117745	0.71(0.17-2.91)	1.000
hepatitis	45/5359	1266/117745	0.84(0.62-1.14)	0.666
hypophysitis	69/5359	1543/117745	1.13(0.88-1.45)	0.797
meningitis	2/5359	241/117745	0.22(0.05-0.87)	0.114
myocarditis	84/5359	1226/117745	1.37(1.09-1.71)	0.033
myositis	67/5359	894/117745	1.44(1.12-1.86)	0.033
neutropenia	69/5359	1285/117745	1.09(0.85-1.40)	0.889
pancreatitis	42/5359	638/117745	1.69(1.23-2.32)	0.015
pericarditis	19/5359	230/117745	1.93(1.20-3.11)	0.033
pleurisy	1/5359	92/117745	0.25(0.03-1.78)	0.467
pneumonitis	225/5359	3007/117745	1.60(1.39-1.84)	<0.001
polyneuropathy	7/5359	184/117745	0.79(0.37-1.69)	0.943
pruritus	84/5359	1980/117745	0.94(0.75-1.17)	0.952
psoriasis	12/5359	310/117745	0.77(0.42-1.40)	0.872
rash	115/5359	3778/117745	0.68(0.56-0.82)	0.001
sjs	3/5359	374/117745	0.18(0.06-0.56)	0.027
thrombocytopenia	83/5359	1607/117745	1.08(0.86-1.36)	0.889
thyroiditis	14/5359	659/117745	0.48(0.28-0.81)	0.033
uveitis	12/5359	394/117745	0.79(0.44-1.40)	0.882
vasculitis	7/5359	129/117745	1.14(0.50-2.60)	1.000
vitiligo	4/5359	310/117745	0.35(0.13-0.95)	0.119

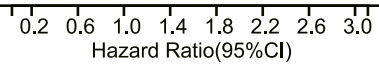

FIGURE 2
The forest plot showing the association between aspirin use and different irAEs among patients receiving immunotherapy. ASA: Aspirin; sjs: Stevens-Johnson syndrome.

However, no significant differences in irAEs were observed in the remaining cancer types (Figure 1).

3.3 Association of aspirin treatment with different irAEs

We conducted a survey to determine the association of aspirin with specific irAEs. Our results revealed that aspirin use was correlated with an elevated risk of several adverse reactions, including anaemia (OR 1.24, 95% CI 1.06–1.47, FDR adjusted p = 0.042) (Figure 2), colitis (OR 1.46, 95% CI 1.27–1.66, FDR adjusted p < 0.001) (Figure 2), myocarditis (OR 1.37, 95% CI 1.09–1.71, FDR adjusted p = 0.033) (Figure 2), myositis (OR 1.44, 95% CI 1.12–1.86, FDR adjusted p = 0.033) (Figure 2), pancreatitis (OR 1.69, 95% CI 1.23–2.32, FDR adjusted p = 0.015) (Figure 2), pericarditis (OR 1.93, 95% CI 1.20–3.11, FDR adjusted p = 0.033) (Figure 2) and pneumonitis (OR 1.60, 95% CI 1.39–1.84, FDR adjusted p < 0.001) (Figure 2). On the other hand, aspirin use was associated with a decreased risk of certain adverse reactions, such as rash (OR 0.68, 95% CI 0.56–0.82, FDR adjusted p = 0.001) (Figure 2), Stevens-Johnson syndrome (OR 0.18, 95% CI 0.06–0.56, FDR adjusted p = 0.027) (Figure 2), and thyroiditis (OR 0.48, 95% CI 0.28–0.81, FDR adjusted p = 0.033) (Figure 2).

3.4 Association of aspirin treatment with irAEs in different organs

Then, we mapped irAEs to their corresponding system organ classes, involving a total of 13 organ systems. Our results demonstrate that aspirin users have a higher risk of developing irAEs in the blood and lymphatic system disorders (OR 1.19, 95% CI 1.06–1.34, FDR adjusted p = 0.019) (Figure 3), cardiac disorders (OR 1.35, 95% CI 1.09–1.66, FDR adjusted p = 0.020) (Figure 3) and respiratory thoracic and mediastinal disorders (OR 1.30, 95% CI 1.12–1.51, FDR adjusted p = 0.004) (Figure 3), while having a lower risk of developing irAEs in the skin and subcutaneous tissue disorders (OR 0.74, 95% CI 0.64–0.86, FDR adjusted p = 0.001) (Figure 3).

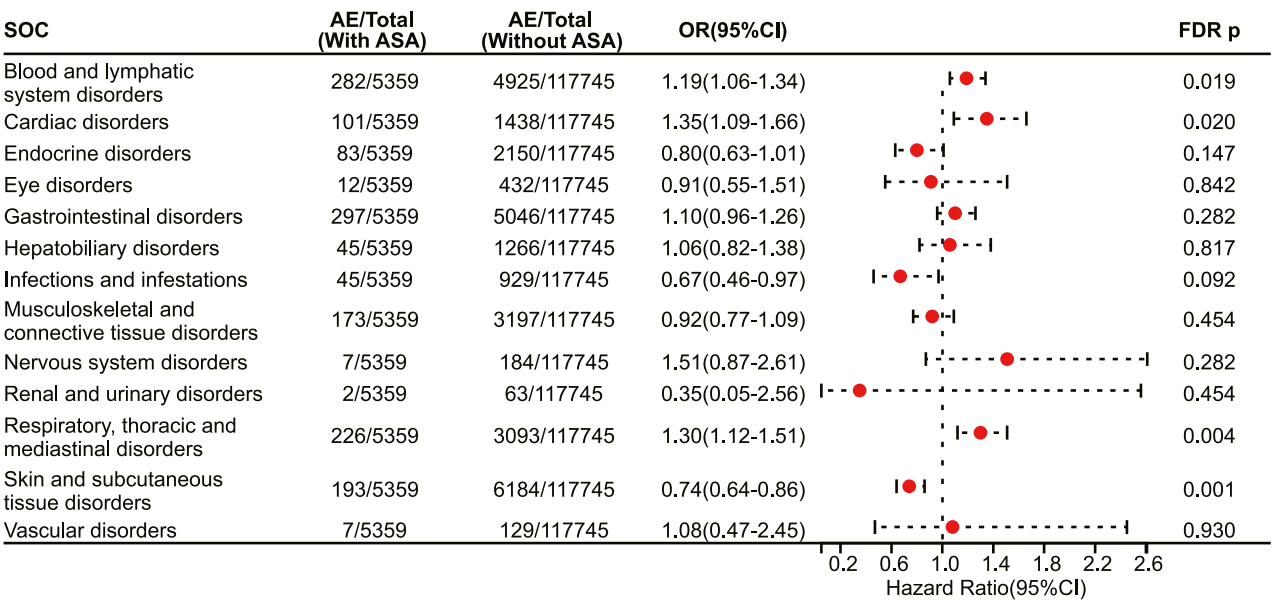

FIGURE 3
The forest plot showing the association between aspirin use and irAEs from different system organ classes (SOC) among patients receiving immunotherapy. ASA: Aspirin.

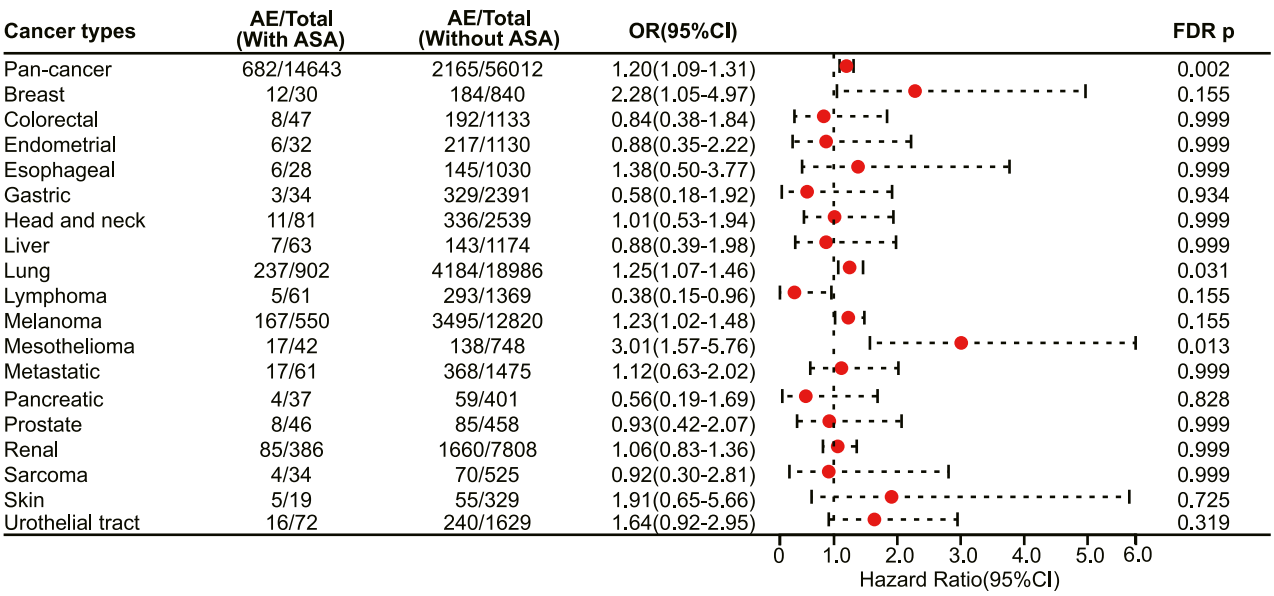

FIGURE 4
The forest plot showing the association between aspirin use and irAEs across different cancer types among patients using PD-1 inhibitors. ASA: Aspirin.

3.5 Association of aspirin use with irAEs among cancer patients treated with PD-1 inhibitors

We next investigated the association between aspirin use and irAE in patients using different ICIs. In a pan-cancer analysis of patients using PD-1 inhibitors, aspirin use was shown to be associated with a higher risk of irAEs (OR 1.20, 95% CI 1.09–1.31, FDR adjusted p = 0.002) (Figure 4). Further analysis revealed that aspirin use was associated with an increased risk of irAEs in lung cancer (OR 1.25, 95% CI 1.07–1.46, FDR adjusted p = 0.031) (Figure 4) and mesothelioma (OR 3.01, 95% CI 1.57–5.76, FDR adjusted p = 0.013) (Figure 4). In addition, for different adverse reactions, the

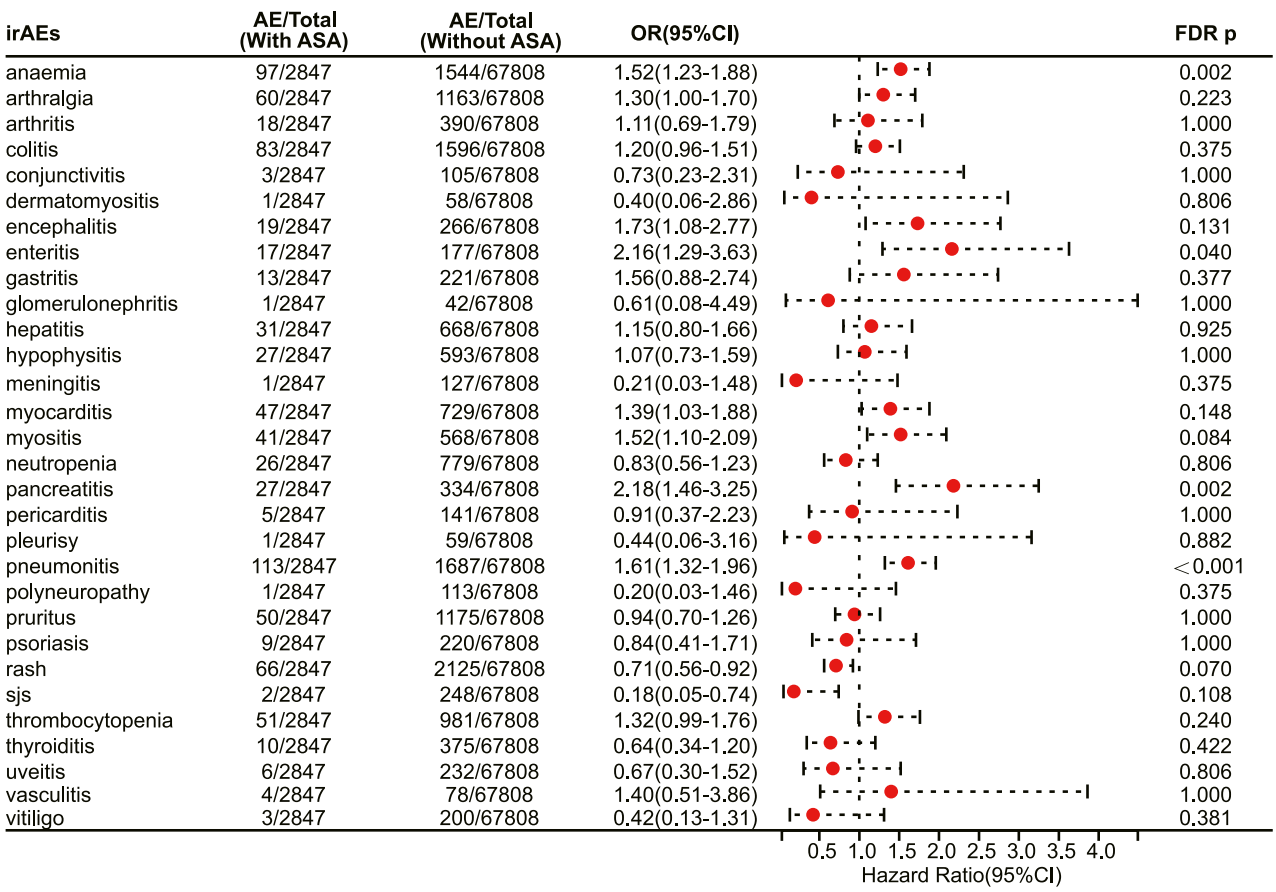

FIGURE 5
The forest plot showing the association between aspirin use and different irAEs among patients using PD-1 inhibitors. ASA: Aspirin; sjs: Stevens-Johnson syndrome.

risk of anaemia (OR 1.52, 95% CI 1.23–1.88, FDR adjusted p = 0.002) (Figure 5), enteritis (OR 2.16, 95% CI 1.29–3.63, FDR adjusted p = 0.040) (Figure 5), pneumonitis (OR 1.61, 95% CI 1.32–1.96, FDR adjusted $p < 0.001$) and pancreatitis (OR 2.18, 95% CI 1.46–3.25, FDR adjusted $p = 0.002$) (Figure 5) were higher in aspirin users.

3.6 Association of aspirin use with irAEs among cancer patients treated with PD-L1 inhibitors

In patients receiving PD-L1 inhibitors, the combination of aspirin demonstrated a tendency to increase adverse reactions in pan-cancer, but there was no statistically significant difference. However, aspirin increased the risk of irAEs in patients with pancreatic cancer (OR 3.48, 95% CI 2.07–5.86, FDR adjusted $p < 0.001$) (Figure 6). In addition, with respect to specific adverse reactions, the risk of colitis (OR 2.31, 95% CI 1.66–3.23, FDR adjusted $p < 0.001$) (Figure 7), pericarditis (OR 4.08, 95% CI 1.93–8.63, FDR adjusted $p = 0.005$) (Figure 7) and pneumonitis (OR 1.57, 95% CI 1.18–2.11, FDR adjusted $p = 0.035$) (Figure 7) were higher in aspirin users.

3.7 Association of aspirin use with irAEs among cancer patients treated with CTLA-4 inhibitors

In patients receiving CTLA-4 inhibitors, there is still a trend towards an increased risk of adverse reactions with the use of aspirin, but only with statistical significance in pancreatic cancer (OR 2.91, 95% CI 1.71–4.96, FDR adjusted $p = 0.002$) (Figure 8). No statistical difference was observed among different immune-related adverse events. Finally, subgroup analysis was not performed for patients receiving LAG-3 inhibitors and PD-1/LAG-3 inhibitors due to the small sample size.

3.8 Hypothetical molecular mechanisms linking aspirin treatment to the risk of irAEs

Until now, the specific mechanisms underlying the association of aspirin with irAEs in cancer patients treated with ICIs remain unknown, but some studies have shown that aspirin plays an important role in immune regulation. Aspirin regulates T cells through COX-1 and COX-2 pathways (Zelenay et al., 2015;

Cancer types	AE/Total (With ASA)	AE/Total (Without ASA)	OR(95%CI)	FDR p
Pan-cancer	239/3844	954/17471	1.17(1.01-1.36)	0.248
Breast	3/25	232/1398	0.73(0.21-2.47)	1.000
Colorectal	4/23	42/382	1.67(0.52-5.39)	1.000
Head and neck	1/20	24/236	0.54(0.07-4.37)	1.000
Liver	9/75	274/2158	1.02(0.47-2.18)	1.000
Lung	91/336	1454/6871	1.44(1.11-1.85)	0.083
Melanoma	4/38	64/296	0.52(0.17-1.56)	1.000
Metastatic	6/29	83/502	1.20(0.43-3.32)	1.000
Ovarian	5/34	114/661	0.97(0.32-2.93)	1.000
Pancreatic	35/107	80/645	3.48(2.07-5.86)	<0.001
Prostate	8/55	37/202	0.75(0.32-1.77)	1.000
Renal	4/52	90/641	0.51(0.18-1.46)	1.000
Skin	1/11	25/223	0.64(0.08-5.28)	1.000
Urothelial tract	12/83	223/1161	0.72(0.38-1.36)	1.000

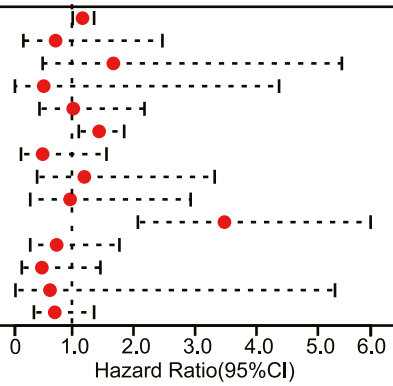

FIGURE 6
The forest plot showing the association between aspirin use and irAEs across different cancer types among patients using PD-L1 inhibitors. ASA: Aspirin.

irAEs	AE/Total (With ASA)	AE/Total (Without ASA)	OR(95%CI)	FDR p
anaemia	36/1193	631/20122	0.88(0.62-1.26)	1.000
arthralgia	11/1193	180/20122	1.19(0.64-2.20)	1.000
arthritis	2/1193	73/20122	0.45(0.11-1.84)	0.802
colitis	43/1193	324/20122	2.31(1.66-3.23)	<0.001
encephalitis	2/1193	121/20122	0.32(0.08-1.29)	0.701
enteritis	3/1193	37/20122	1.93(0.58-6.39)	0.802
gastritis	2/1193	39/20122	1.04(0.25-4.40)	1.000
hepatitis	5/1193	154/20122	0.61(0.25-1.49)	0.802
hypophysitis	4/1193	55/20122	1.40(0.50-3.93)	1.000
myocarditis	15/1193	178/20122	1.38(0.81-2.35)	0.802
myositis	12/1193	122/20122	1.48(0.79-2.77)	0.802
neutropenia	31/1193	323/20122	1.55(1.06-2.27)	0.275
pancreatitis	3/1193	106/20122	0.53(0.17-1.69)	0.802
pericarditis	9/1193	40/20122	4.08(1.93-8.63)	0.005
pneumonitis	57/1193	611/20122	1.57(1.18-2.11)	0.035
polyneuropathy	5/1193	28/20122	2.65(1.01-6.97)	0.428
pruritus	5/1193	151/20122	0.59(0.24-1.46)	0.802
psoriasis	3/1193	50/20122	0.99(0.31-3.22)	1.000
rash	10/1193	309/20122	0.64(0.34-1.20)	0.802
sjs	1/1193	52/20122	0.40(0.06-2.92)	0.912
thrombocytopenia	16/1193	383/20122	0.68(0.41-1.13)	0.777
uveitis	1/1193	21/20122	0.90(0.12-6.76)	1.000
vasculitis	3/1193	18/20122	1.54(0.35-6.73)	1.000

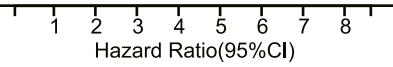

FIGURE 7
The forest plot showing the association between aspirin use and different irAEs among patients using PD-L1 inhibitors. ASA: Aspirin; sjs: Stevens-Johnson syndrome.

Rachidi et al., 2017), and activated T cells may lead to increased irAEs risk (Khan and Gerber, 2020). In addition, the modulation of gut microbiota by aspirin may also mediate the increased risk of irAEs (Chaput et al., 2017) (Figure 9).

4 Discussion

By understanding how the immune system interacts with tumor cells, scientists have established new therapies for cancer treatment that have brought noteworthy clinical benefits for cancer patients (Morad et al., 2021). However, many cancer patients have underlying diseases, and the presence of other drugs may affect the immunotherapy. ICIs leverage diverse mechanisms and pathways to harness the immune system's ability to eradicate tumor cells. Consequently, potential interactions between concomitant medications and ICIs transcend the typical assessment of pharmacodynamic and pharmacokinetic interactions between drugs. Aspirin is currently one of the most widely used basic drugs, Previous studies reported that aspirin use is

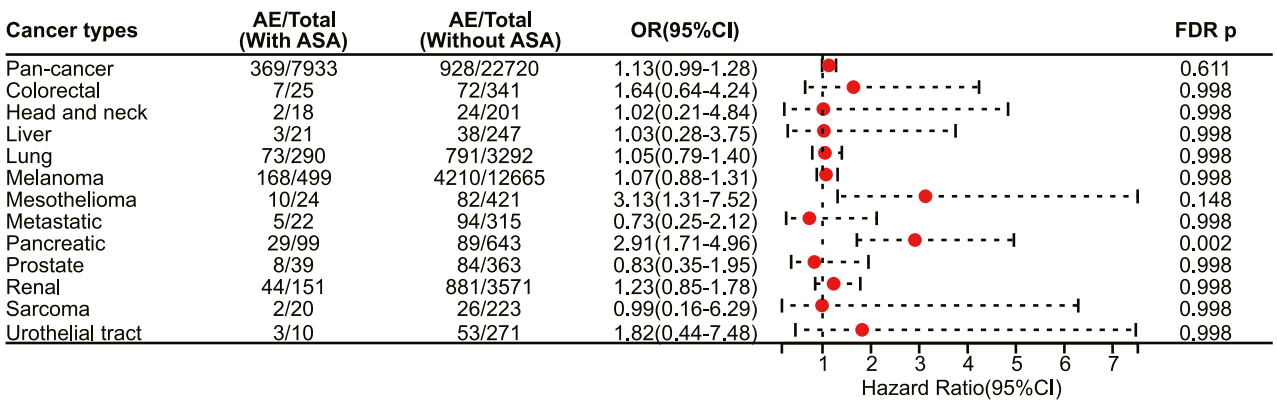

FIGURE 8
The forest plot showing the association between aspirin use and irAEs across different cancer types among patients using CTLA-4 inhibitors. ASA: Aspirin.

associated with better outcomes with immunotherapy, However, it is not clear whether aspirin use impacts irAEs. This is one of the first studies to analyze the association between aspirin use with irAEs using FEARS data with innovative and comprehensive benefits. Our study showed that aspirin exposure was associated with an increased risk of irAEs in all enrolled cancer patients treated with ICIs. However, it is important to note that the relationship between aspirin use and irAE risk varies across different tumor types, types of irAEs, and various ICIs. Our research findings highlight these distinctions.

As a well-known non-selective COX inhibitor, aspirin irreversibly acetylates the active sites of COX-1 and COX-2, thereby reducing their enzyme activity (Ornelas et al., 2017). COX-1 mainly mediates the formation of physiological prostaglandins, such as Thromboxane A2 (TXA2), which in turn promotes platelet aggregation (Menter and Bresalier, 2023). Aspirin inactivates COX-1 and prevents the production of TXA2, thus acting as an antiplatelet and preventing thrombosis. More importantly, previous studies have confirmed the role of platelets in promoting tumor growth and metastasis (Lichtenberger and Vijayan, 2019). Rachidi et al. (2017) found that a protein called Glycoprotein A repetitions predominant (GARP) exists on the surface of platelets, which traps and activates Transforming growth factor-β (TGF-β). TGF-β is an immunomodulatory molecule that suppresses CD4 and CD8 T cells, allowing tumors to evade the immune system. Riesenberg et al. (2019) confirmed through a mouse model that the antiplatelet effect of aspirin can inhibit TGF-β signaling, thereby enhancing T cell function, and synergistically exerting anti-tumor effects with PD-1 blocker.

COX-2 is an inducer of enzymes that promote the synthesis of inflammatory prostaglandins, such as Prostaglandin E2 (PGE2), which can cause inflammation (Jin et al., 2023). Interestingly, PGE2 has been shown to regulate the function of various immune cells within the tumor microenvironment (TME), including myeloid-derived suppressor cells (MDSCs), dendritic (DC) cells, natural killer (NK) cells, CD4 and CD8 T cells, resulting in immune evasion (Zelenay et al., 2015; Böttcher et al., 2018; Bonavita et al., 2020). Moreover, PGE2 is capable of upregulating PD-L1 expression (Goto et al., 2020) and inhibit T cell receptor activation (Newick et al., 2016). The above study suggests that aspirin may exert immunomodulatory effects and enhance T cell activation by inhibiting COX2/PGE2 pathway (Wei et al., 2022; Jin et al., 2023).

Aspirin has been found to aid ICIs in breaking immune tolerance and amplifying the immune response (Zelenay et al., 2015). Unfortunately, it is important to note that immune activation is not limited to tumor-specific responses. Some researchers have proposed that activated effector T cells also attack normal non-tumor tissues while increasing their anti-tumor activity (Khan and Gerber, 2020; Ronen et al., 2022). T-cell receptor (TCR) sequencing studies have provided evidence to support this theory (Porciello et al., 2022; Sanromán Á et al., 2023). In patients treated with Ipiliumumab, researchers detected greater CD4 and CD8 T cell diversity in irAEs patients compared with those who did not experience significant adverse reactions (Oh et al., 2017). A recent work from Luoma and others has demonstrated the presence of a large number of CD8 T cells with high cytotoxicity and proliferation ability in the colon of patients with colitis, and these CD8 T cells are mostly from tissue-resident populations (Luoma et al., 2020). Together, these studies support that irAEs may be caused by the mobilization of a large number of T cells (Ramos-Casals et al., 2020). Other studies have shown that the presence of cross-antigens can also influence T-cell responses. In a study by Berner et al., 73 patients with NSCLC who received anti-PD-1 treatment were included, and nine common T-cell antigens were identified between tumor tissues and skin. This indicates that ICIs target both non-small-cell lung cancer (NSCLC) cancer and skin, leading to immune-related dermal toxicity while treating tumors (Berner et al., 2019). On the other hand, self-antigens from dying cells are captured by antigen-presenting cells (APCs) during tumor cell killing. These APCs then migrate to lymph nodes and activate more reactive T and B cells, These novel T cell clones may initiate a distinct immuno-editing wave, leading to adverse reactions (Yost et al., 2019; Baumjohann and Brossart, 2021).

Multiple clinical studies have investigated the potential of aspirin in enhancing the immune response in immunotherapy. Cortellini et al. (2020) reported that concurrent use of aspirin

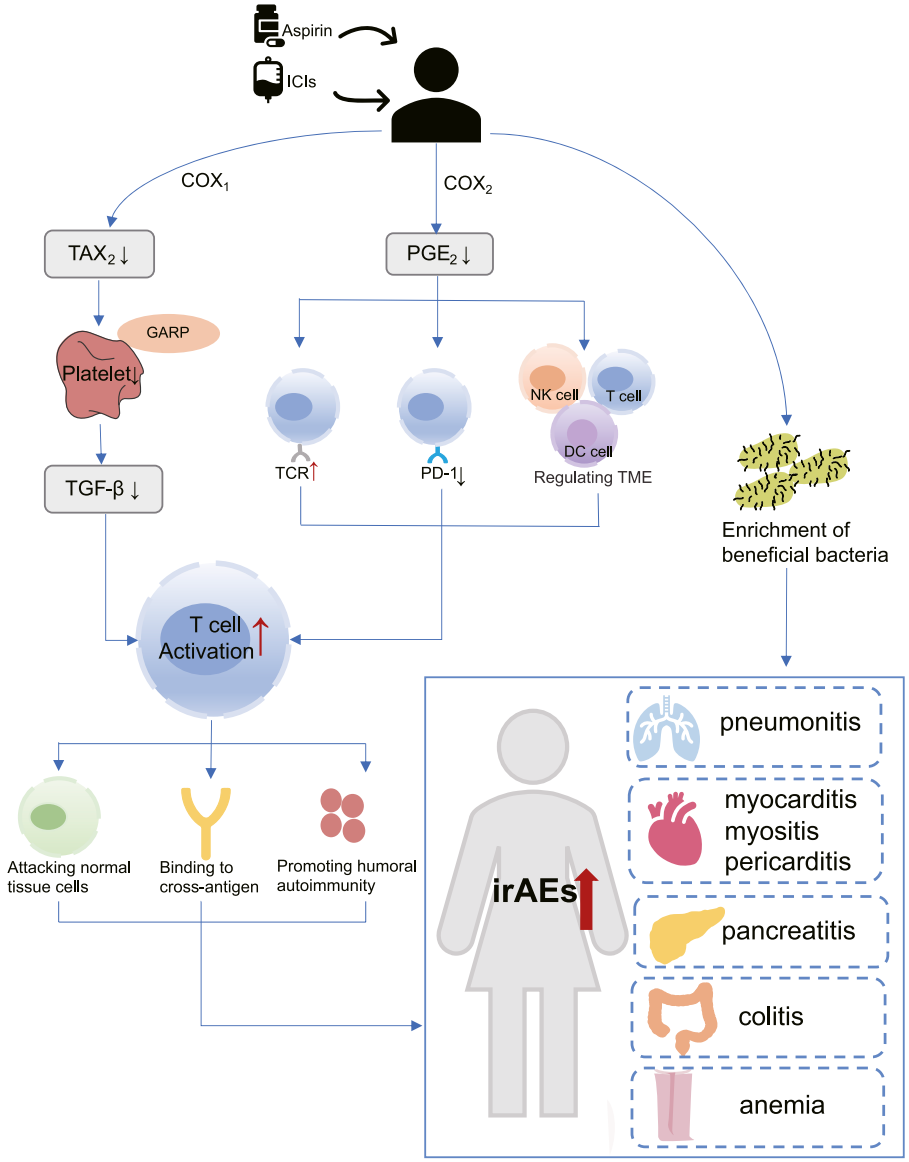

FIGURE 9
The hypothetical mechanism of increased risk of immune adverse reactions with aspirin use in patients receiving immunotherapy. COX-1: Cyclooxygenase-1; COX-2: Cyclooxygenase-2; DC cell: Dendritic cell; GARP: Glycoprotein A repetitions predominant; ICIs: Immune checkpoint inhibitors; irAEs: Immune-related adverse events; NK cell: Natural killer cell; PGE2: Prostaglandin E2; PD-1: programmed cell death-1; TAX2: Thromboxane2; TGF-β: Transforming growth factor-β; TCR: T-cell receptor; TME: tumor microenvironment.

can improve overall response rate (ORR) among patients with solid tumors receiving PD-1/PD-L1 checkpoint inhibitors. Another study have highlighted that aspirin can prolong overall survival (OS) (Kostine et al., 2021). Furthermore, a meta-analysis suggested a significant intensification in progression-free survival (PFS) with concurrent use of aspirin and ICIs (Zhang et al., 2021). The above statements have demonstrated the synergistic effect of aspirin in ICIs. Therefore, aspirin may have underestimated immunomodulatory effects can amplify immune activation induced by ICIs. However, coins always have two sides. Over-activated T cells lack tumor specificity, so we have to consider the impact of aspirin on irAEs. We propose that aspirin may enhances T cell activation through inhibition of PGE2 and platelets, contributing to the increased irAEs.

Moreover, it has been shown that microbiota composition was a key factor in maintaining immune homeostasis, and may affect the occurrence of irAEs (Dora et al., 2023). Chaput et al. (2017) demonstrated that protective bacteria in the gut led to positive outcomes for patients who receive ipilimumab therapy, but also with a higher incidence of ipilimumab-induced colitis. Mouse models have shown that aspirin modulates the gut microbiota by enrichment of probiotics (Zhao et al., 2020; Brennan et al., 2021). This may also be one of the reasons why aspirin is associated with an increased risk of irAEs occurring (Figure 9).

Our research has uncovered a connection between the use of aspirin and an increased susceptibility to irAEs in pan-cancer patients. Delving deeper into our findings, we have identified a notably increased risk of irAEs among patients afflicted with specific cancer types, including lung cancer, mesothelioma, and pancreatic cancer. Conversely, a perplexing reduction in irAE risk has emerged in lymphoma patients. Remarkably, these observations constitute a novel contribution to the field, as they have not been previously documented in existing literature.

In stark contrast to prior retrospective studies, our comprehensive analysis has demonstrated robust statistical significance in support of these findings (Gandhi et al., 2020; Sieber et al., 2022). We posit that aspirin's influence on the occurrence of irAEs may be mediated through the COX pathway, thereby shedding light on a potential mechanistic explanation. Furthermore, the intriguing divergence observed within the lymphoma subgroup warrants further investigation. While our data show a diminished risk of irAEs in lymphoma patients, it is essential to acknowledge that this subgroup comprises a relatively small sample size, constituting only 1.5% of the overall study population. It is conceivable that this statistical anomaly may be attributed to the limited representation of lymphoma cases, or it may signify the existence of hitherto undiscovered mechanisms that demand further exploration and scrutiny. In addition, aspirin use, prescribing status, or combination of aspirin with these conditions. These circumstances will also have an impact on our results (Colard-Thomas et al., 2023).

Our in-depth analysis revealed a significant association between the use of aspirin and a range of irAEs. Specifically, we observed that aspirin use markedly increased the risk of patients experiencing irAEs such as pneumonia, myocarditis, myositis, pericarditis, pancreatitis, colitis, and anemia. In contrast, the risk of irAEs related to conditions like rash, Stevens-Johnson syndrome, and thyroiditis was notably reduced. To further support our conclusions, we conducted a comprehensive review of previously published articles, seeking evidence that aligns with the associations we identified. Prior studies may not have fully considered the relationship between aspirin and irAEs or may not have detected these associations due to differences in research methodologies. Nonetheless, our study fills this knowledge gap and provides healthcare professionals with a more comprehensive understanding of aspirin's role in irAE risk.

In summary, these findings underscore the need for heightened vigilance among clinicians when treating patients with immunotherapy, especially in cases related to irAEs affecting organs or systems such as the gastrointestinal tract, lungs, pancreas, heart, and anemia. However, it is also essential to consider an additional factor, namely, the widespread use of aspirin in cardiovascular disease treatment (Byrne and Colleran, 2020), where a patient's history of cardiovascular conditions may be one of the factors contributing to the heightened risk of irAEs (Yousif et al., 2023). Therefore, a comprehensive assessment of the patient's overall health and treatment needs is crucial.

Despite some limitations in our study and a lack of supporting mechanistic research, our research still provides valuable pharmacological guidance to the greatest extent possible. For example, when using aspirin in patients receiving PD-1 inhibitors, it is advisable to pay closer attention to indicators related to anemia, enteritis, pneumonia, and pancreatitis. Similarly, for patients undergoing PD-L1 inhibitor treatment, increased attention should be directed towards indicators associated with colitis, pericarditis, and pneumonia. Furthermore, in patients receiving CTLA-4 inhibitors, no association has been observed between aspirin and irAEs, although further research is needed to confirm this, in order to offer clinicians more precise treatment guidelines.

Overall, our study highlights the potential risks associated with aspirin use in patients receiving immunotherapy, particularly with regards to irAEs. These findings could inform clinical decision-making and improve patient safety.

5 Study limitations

The FAERS database, as a voluntary, passive, and non-mandatory reporting system, faces inherent challenges. These include incompleteness, inaccuracy, inconsistency, and delay in reporting adverse events. These limitations stem from various factors, primarily the lack of detailed patient characteristics, drug exposure information, and outcome details, such as the dose and duration of aspirin use, as well as whether patients received other treatment regimens and the sequence of medication. These factors may influence the associations observed and the study outcomes. Therefore, it is essential to carefully consider these limitations, particularly when interpreting the research results.

Furthermore, our analysis is influenced by the uneven distribution of cases within the database, with a higher number of lung cancer patients but significantly fewer patients with other cancer types. This non-uniform case distribution may introduce bias and restrict the generalizability and applicability of our study findings.

To overcome these limitations and provide more robust insights, further prospective clinical studies are urgently needed. Additionally, the mechanisms underlying the association between aspirin use and irAEs remain unclear, underscoring the need for fundamental research to address these uncertainties and advance our understanding of immunotherapy.

6 Conclusion

This study has revealed a significant association between aspirin usage and irAEs in cancer patients undergoing ICIs. It is important to note that this association exhibits variations depending on the specific cancer type, the nature of adverse events, and the specific type of ICIs being utilized. These findings underscore the importance of assessing the effect of baseline drugs, including aspirin, on the safety and efficacy of ICIs in tumor treatment, and tailoring treatment plans accordingly on an individual basis.

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

HY: Data curation, Formal Analysis, Investigation, Project administration, Resources, Validation, Visualization, Writing–original draft, Writing–review and editing. ZL: Investigation, Methodology, Writing–review and editing. RL: Methodology, Resources, Validation, Visualization, Writing–review and editing. RH: Data curation, Formal Analysis, Investigation, Resources, Software, Validation, Visualization, Writing–review and editing. XP: Conceptualization, Funding acquisition, Methodology, Supervision, Writing–review and editing.

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Conflict of interest

Author RH was employed by Hangzhou Linan Guorui Health Industry Investment Co., Ltd.

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

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Adverse events of immune checkpoint therapy alone versus when combined with vascular endothelial growth factor inhibitors: a pooled meta-analysis of 1735 patients

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Background: Combining immune checkpoint therapy (ICT) and vascular endothelial growth factor inhibitors (VEGFi) may result in increased treatment-related and immune-related adverse events (TRAEs and irAEs) compared to ICT alone. This metanalysis was conducted to identify prospective phase II or III clinical studies that evaluated the toxicity profile of ICT + VEGFi compared to ICT alone.

Methods: A systematic search was performed across all cancer types and major databases until August 10, 2022, and screening was done by two independent investigators. Inclusion criteria included phase 2 or 3 studies with at least one arm of patients treated with combination therapy and one arm treated with monotherapy. Adverse event data were pooled using a restricted maximum likelihood fixed effects model, and heterogeneity using Cochran's Q (chi-square) test.

Results: 7 out of 9366 studies met the inclusion criteria, and 808 and 927 patients were treated with ICT monotherapy and a combination of ICT with VEGFi, respectively. Only one study reported irAEs, so the analysis was restricted to TRAEs. The total number of TRAEs was significantly higher in the ICT + VEGFi group (RR:1.49; 95% CI 1.37 -1.62; p=1.5×10-21), and more frequent treatment withdrawals were attributed to TRAEs (RR:3.10; 95% CI 1.12-8.59; p=0.029). The highest TRAE effect size increases noted for rash (RR 6.50; 95% CI 3.76 – 11.25; p=2.1×10-11), hypertension (RR:6.07; 95% CI 3.69–10.00; p=1.3×10-12), hypothyroidism (RR:5.02; 95% CI 3.08 – 8.19; p=8.9×10-11), and diarrhea (RR:4.94; 95% CI 3.21–7.62; p=3.8×10-13). Other significantly more frequent TRAEs included nausea, anemia, anorexia, and proteinuria.

Conclusion: Combination therapy with ICT and VEGFi carries a higher risk of certain TRAEs, such as rash, hypertension, hypothyroidism, diarrhea, nausea, anorexia, and proteinuria, compared to ICT monotherapy. More granular details on the cause of AEs, particularly irAEs, should be provided in future trials of such regimens.

KEYWORDS

cancer, immunotherapy, toxicity, adverse events, immune checkpoint inhibitor, vascular endothelial - growth factor

Background

Immune checkpoint inhibitors therapy (ICT) combined with vascular endothelial growth factor inhibitors (VEGFi) are now established standard of care regimens across diverse malignancies such as renal cell carcinoma, endometrial cancer and hepatocellular carcinoma (1–4). However, there is limited knowledge about the potential synergistic toxicities between combination of ICT with VEGFi when compared to ICT alone (5–9). Prior studies have aimed to describe the general safety profile of this combination. For example, Tao et al. conducted a meta-analysis to evaluate the efficacy and toxicity of ICT and VEGFi in comparison to VEGFi alone in patients with renal cell carcinoma (RCC). The study included six randomized clinical trials (RCTs) and analyzed dose-limiting adverse events (AEs) related to RCC treatment. The results showed an increased risk of any-grade treatment-related adverse events (TRAEs), such as hypertension, arthralgia, and proteinuria, in the combination group compared to the VEGFi alone group. However, the risk of some TRAEs, such as hand-foot skin reaction (HFSR) [RR = 0.47, 95% CI: 0.28– 0.79], stomatitis (RR = 0.71, 95% CI: 0.56–0.91), and dysgeusia (RR = 0.42, 95% CI: 0.26–0.68), was lower in the combination group (1). A similar study by He et al. included six RCTs and evaluated the safety and efficacy of ICT and VEGFi combination therapy compared to VEGFi alone in the treatment of RCC. The results showed no significant difference in grade 3 or higher TRAEs between the two groups (2). A small study by Rizzo et al. aimed to evaluate the risk of gastrointestinal (GI) toxicities of a combination of immunotherapy with tyrosine kinase inhibitors (TKIs) compared to sunitinib alone. The meta-analysis of four RCTs showed an increased risk of selected GI TRAEs, such as diarrhea and decreased appetite, in the combination group, while the risk of nausea was higher in the sunitinib group (3). Several early-phase trials and retrospective studies also aimed to look at the efficacy and toxicity of ICT and TKIs, such as epidermal growth factor receptor inhibitors (EGFRi), as a combination therapy as well as sequential therapy. These studies results exhibited discrepancy with some of them suggesting increased toxicity of combination and sequential therapies while others reported acceptable safety profiles (10–13). Discrepant results from these studies create a knowledge gap regarding the risk of added toxicities of ICT/VEGFi combination regimens. Moreover, the results of meta-analyses by Abdelhafeez et al. and Da et al. showed an expected increase overall risk of irAEs with the use of two combined immune checkpoint inhibitors as compared to one (14, 15). However, little is known regarding the risk of immune- related AEs (irAEs) of ICT/VEGFi combination therapy. Our hypothesis was that there are added immune and other toxicities when using combination of ICT/VEGFi as compared to ICT alone. To answer this question, we conducted a meta-analysis of reported or published studies with toxicity data of ICT/VEGFi combination therapy as compared to ICT alone.

Methods

Data sources and search strategies

We performed a systematic search in Ovid MEDLINE, Ovid Embase, Clarivate Web of Science and Wiley Cochrane Library from the inception of the databases to August 10, 2022. Search structures, subject headings, and keywords were tailored to each database by a medical research librarian (YG). The following concepts were searched using subject headings and keywords as needed, "cancer", "neoplasm", "immunotherapy", "checkpoint inhibitor", "cytotoxic t- lymphocyte-associated antigen 4", "programmed cell death 1", "programmed cell death ligand 1", "vascular endothelial growth factor", "VEGF inhibitor", "vascular endothelial growth factor receptor", "anti-vascular", "anti-VEGF", "anti-angiogenic", "angiogenesis inhibitor", "ipilimumab", "tremelimumab", "pembrolizumab", "nivolumab", "spartalizumab", "cetrelimab", "atezolizumab", "durvalumab", "avelumab", "cemiplimab", "monalizumab", "aflibercept", "bevacizumab", "ranibizumab", "brolucizumab", "conbercept", "pazopanib", "sunitinib", "sorafenib", "regorafenib", "cabozatinib", "lenvatinib","ponatinib", "axitinib", "tivozanib", "ramucirumab", "vandetanib", and "sitravatinib". The search terms were combined by "or" if they represented the similar concept, and by "and" if they represented different concepts. Database search strategies are detailed in the Supplementary Tables S1–S4.

Eligibility criteria

In determining eligibility for our review, we established several inclusion criteria. The studies had to be phase 2 or 3, reported in English, and include at least one arm of adult patients treated with a

combination of ICT and VEGFi, as well as one arm treated with ICT monotherapy. Additionally, the studies had to report outcomes related to TRAEs and/or IRAEs. We excluded non-comparative and non-original studies, and studies that did not report AEs. Retrospective studies were also excluded from our analysis because we aimed to ensure the integrity and reliability of our data in relation to CTCAE criteria. By using prospectively collected data with CTCAE criteria, we aimed to minimize the potential for recall bias, which can occur when relying on retrospective data. Abstracts without a full text that met our inclusion criteria were still included in the analysis.

Study selection

The study selection process was carried out by two independent reviewers (IK and LW) who screened all titles and abstracts based on the defined inclusion and exclusion criteria. The full text of relevant references were obtained and evaluated by the same two reviewers. In case of any discrepancy in selection, a third reviewer (OA) was involved to resolve it.

Data extraction

Data extraction was performed by two independent reviewers (IK and LW) using Microsoft Excel. Any discrepancies in data extraction were resolved by two other independent reviewers (OA and RB). The following variables were collected from each study: study characteristics, participant characteristics, intervention details, and the outcomes of interest, which included the total sample size and the number of events in each group.

Outcomes of interest

The outcomes of interest included treatment-related adverse events (TRAEs) and immune-related adverse events (irAEs). The categorization of TRAEs and irAEs was predicated upon definitions provided by the individual studies included. When a study explicitly defined an event as an irAE, we categorized the data accordingly. In the absence of such specific categorization, events were defaulted to treatment related. This methodology ensured consistency and minimized interpretative biases in our analysis. TRAEs included symptoms such as diarrhea, rash, HFSR, fever, dry mouth, pruritus, conjunctivitis, hypomagnesemia, dysphonia, nausea, increased creatinine, increased ALT, increased AST, increased bilirubin, increased lipase, fatigue, asthenia, hypertension, anorexia, weight loss, mucositis, decreased platelet count, thyroid dysfunction, thyroiditis, hypothyroidism, anemia, increased TSH, decreased lymphocytes, decreased neutrophils, headache, infection, proteinuria, arthralgia, seizures, hyperglycemia, infusion reaction, and more. Similarly, irAEs included symptoms such as abdominal pain, increased ALP, increased ALT, increased AST, increased bilirubin, cerebral edema, colitis, conjunctivitis, increased creatinine, arthralgia, diarrhea, dyspnea, hypothyroidism, hyperthyroidism, infusion reaction, hyperglycemia, myalgia, rash, and others. The Common Terminology Criteria for Adverse Events v3.0 was used to grade the adverse events. The events were considered for analysis if they were reported similarly by at least two studies.

Quality assessment

The methodologic quality was assessed using the Cochrane risk-of-bias tool for randomized trials. The risk of bias was only assessed for published full-length articles. Two reviewers independently (IK and LW) assessed trial quality of studies by examining several components: randomization process, deviations from intended interventions, missing outcome data, selective reporting, funding and any other potential source of bias. Any conflicts were resolved by consensus. The quality of the studies is represented in the Table 1.

Data analysis

For the adverse events, we calculated relative risk along with 95% confidence intervals and we pooled the effect estimates across the studies following the restricted maximum likelihood fixed heterogeneity. For the assessment of heterogeneity, we used Cochran's Q (chi square) test, P value <0.1 is considered statistically significant and I 2 ≥ 50% suggested substantial heterogeneity. Forest plots were constructed to illustrate the

TABLE 1 Traffic light plot showing the risk of bias of the two completed studies.

Author, Year	Risk of bias domains						
	D1	D2	D3	D4	D5	D6	D7
Nayak, 2020 (16)	Low	Low	Low	Low	Some Concerns	Low	Some Concerns
Lheureux, 2020 (17)	Low	Low	Low	Low	Some Concerns	Low	Low

Domains:
D1: Overall ROB.
D2: ROB from randomization process.
D3: ROB due to deviations from intended interventions.
D4: ROB due to missing outcome data.
D5: ROB in measurement of outcomes.
D6: ROB in selection of the reported results.
D7: Other (funding, conflict of interest).

results of the meta- analysis. Statistical analyses were completed using R version 4.2.2 (R Core Team, 2020).

Ethics statement

This study was exempt from Institutional Review Board review as it involved the analysis of existing publicly available data.

PRISMA statement

This study was conducted in accordance with the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). The PRISMA checklist was used to guide the study and is available upon request (Table S5 in the appendix) (18, 19).

Results

Study characteristics

A total of 11,130 potential titles and abstracts were identified through the electronic search strategy, with 32 duplicates removed internally and 1764 duplicates removed through the assistance of a medical research librarian (YG). The remaining 9366 studies underwent primary screening, and 721 full-text articles or abstracts were evaluated for eligibility (as shown in Figure 1) (19)]. After secondary screening, seven studies were included in the analysis, involving a total of 808 patients treated with ICT monotherapy and 927 patients treated with a combination of ICT and VEGFi. The characteristics of the included studies are summarized in Table 2. Further details on the baseline characteristics of the studies can be found in Table S6 in the appendix.

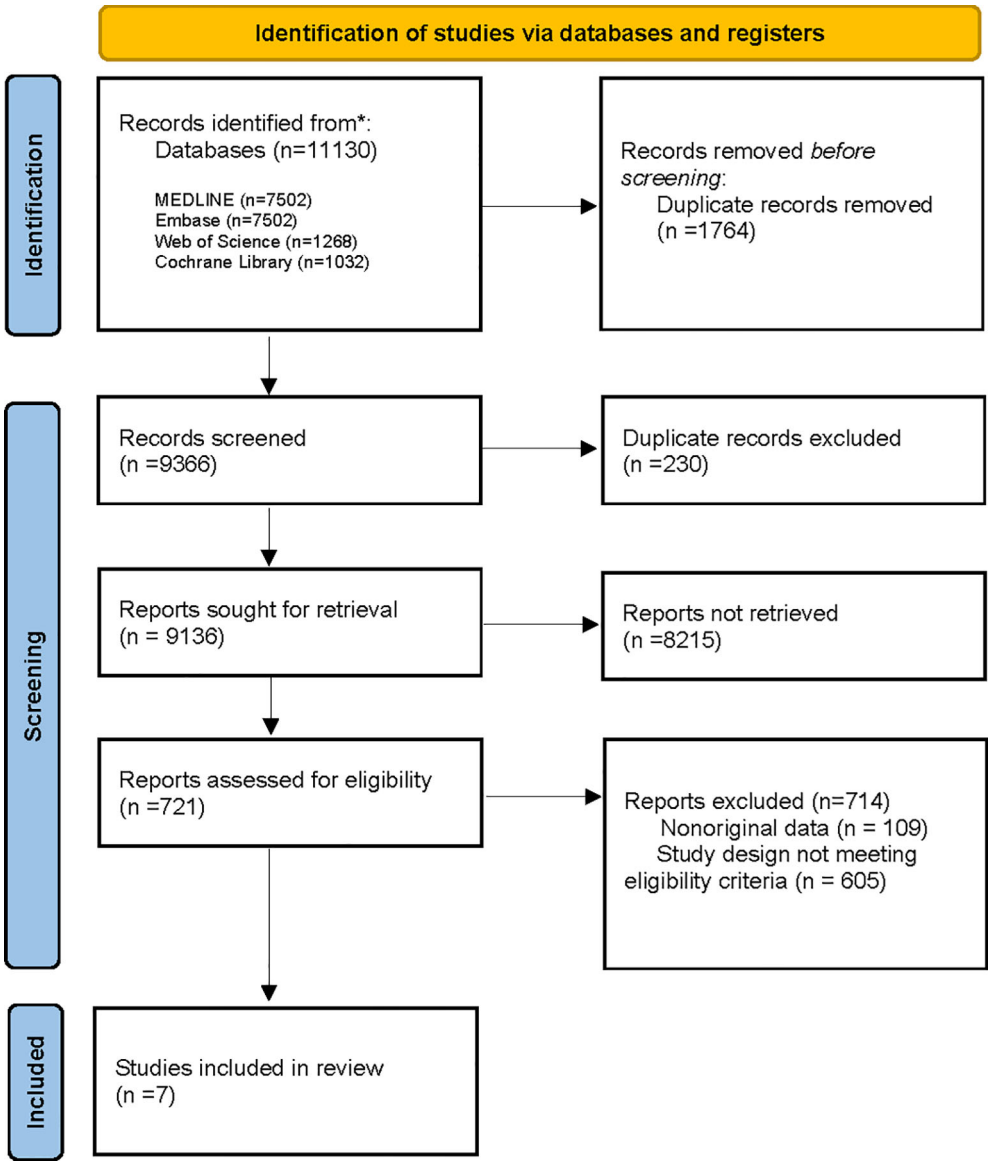

FIGURE 1
Flowchart demonstrating the process of study selection (7).

TABLE 2 Baseline characteristics of patients among the included studies.

Year	Author	Trial name	NCT number	Phase	Conference	Full manuscript (FM) vs Abstract (A)	Number of patients	Median age	Males, %	Cancer type	ICT arm	ICT + VEGFi arm
2018	Bendell et al. (20)	IMblaze370	NCT02788279	3		FM	363	58		colorectal	atezolizumab	Atezolizumab + cobimetinib
2017	McDermott et al. (21)	IMmotion150	NCT01984242	2	2017 Genitourinary Cancers Symposium	A	305			RCC	atezolizumab	Atezolizumab + bevacizumab
2020	Lonardi et al. (22)	CARACAS	NCT03944252	2	2020 ASCO Annual Meeting	A	60	63	31.6	Squamous cell anal carcinoma	avelumab	Avelumab + cetuximab
2022	Loriot et al. (23)	LEAP-011	NCT03898180	3	2022 ASCO Genitourinary Cancers Symposium	A	441			Urothelial carcinoma	pembrolizumab	Pembrolizumab + lenvatinib
2020	Nayak et al. (16)		NCT02337491	2		FM	80	53	67.5	Glioblastoma	pembrolizumab	Pembrolizumab + bevacizumab
2021	Yang et al. (24)	LEAP-007	NCT03829332	3	The ESMO Immuno-Oncology Congress 2021	A	623	66		Non-small cell lung cancer	pembrolizumab	Pembrolizumab + lenvatinib
2020	Lheureux et al. (17)		NCT03367741	2	2020 ASCO Annual Meeting	A	82			Endometrial carcinoma	nivolumab	Nivolumab + carbozatinib

ICT, immune checkpoint inhibitors therapy; VEGFi, vascular endothelial growth factor inhibitors.

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Adverse events

Only one of the studies reported immune-related adverse events (irAEs). As a result, the analysis was restricted to treatment-related adverse events (TRAEs). The TRAEs included in the analysis were anemia, anorexia, diarrhea, fatigue, hypertension, hypothyroidism, lymphopenia, nausea, proteinuria, pruritus, and rash of any grade. The total number of TRAEs was significantly higher in the group receiving the combination of ICT and VEGFi (relative risk [RR] 1.49; 95% CI 1.37 - 1.62; p=1.5×10-21). The rate of grade 5 TRAEs was higher in the combination group (RR 2.86; 95% CI 1.29 - 6.31; p=0.0091). Treatment withdrawals due to TRAEs were also higher in the combination group (RR 3.10; 95% CI 1.12 - 8.59; p=0.029). However, the signal for an increased rate of treatment interruptions due to TRAEs was weaker (RR 1.28, 95% CI 0.99 - 1.65; p=0.057). The increased risk of TRAEs was significantly higher in the combination group for the following events: anorexia (RR 2.49; 95% CI 1.45 – 4.30; p=9.5×10-4), diarrhea (RR 4.94; 95% CI 3.21 – 7.62; p=3.8×10-13), hypertension (RR 6.07; 95% CI 3.69 – 10.00; p=1.3×10-12), hypothyroidism (RR 5.02; 95% CI 3.08 – 8.19; p=8.9×10-11), nausea (RR 3.10; 95% CI 1.93 – 5.00; p=3.1×10-6), proteinuria (RR 2.15; 95% CI; p=3.6×10-6), and rash (RR 6.50; 95% CI 3.76 – 11.25; p=2.1×10- 11). However, the risk was inconclusive for certain TRAEs such as anemia (RR 3.00, 95% CI 0.66 - 13.45; p=0.15), lymphopenia (RR 1.08, 95% CI 0.27 - 4.23; p=0.9), fatigue (RR 1.18, 95% CI 0.82 - 1.69; p=0.35), and pruritus (RR 0.70, 95% CI 0.43 - 1.13; p=0.15). The list of TRAEs and their corresponding effect sizes can be found in Table 3. Forest plots demonstrating our results are presented in Figures 2–6.

Discussion

This systematic review and meta-analysis evaluated the safety of combining ICT with VEGFi compared with ICT alone in adult patients with cancer, incorporating data from 7 studies with a total population of 1735 patients. To the best of our knowledge, this is the first systematic review and meta-analysis that compared toxicity of a combination of ICT with VEGFi as compared to ICT monotherapy across various cancer types. Our study showed that the combination therapy was associated with a significantly increased risk of treatment-related toxicity, risk of death, and treatment discontinuation due to adverse events (AEs). The results demonstrated that the combination therapy increased the risk of anorexia, diarrhea, hypertension, hypothyroidism, nausea, proteinuria, and rash. These AEs are also commonly encountered in monotherapies with VEGFi (25, 26).

The impact of combining ICT with VEGFis on the incidence of adverse events has been a subject of heightened interest. Our findings show a notable association between the two, which is in alignment with the COSMIC-312 trial results. In this pivotal trial

TABLE 3 Summary of the safety findings of the combination of ICT with VEGFi versus ICT alone.

Outcome	Number of studies	Total combined	Total events combined	Total individual	Events individual	RR	95% CI	P value
Total % of TRAEs	5	694	503	688	332	1.49	1.37 -1.62	1.5×10^{-21}
Grade 3-4 TRAEs	4	538	253	434	89	2.40	1.93 - 2.97	1.7×10^{-15}
Grade 5 TRAEs	4	678	22	670	7	2.86	1.29 - 6.31	9.1×10^{-3}
Treatment interruption due to TRAEs	5	435	107	404	77	1.28	0.99 - 1.65	5.7×10^{-2}
Treatment withdrawal due to TRAEs	3	167	16	151	4	3.10	1.12 - 8.59	2.9×10^{-2}
Any grade anemia	2	66	9	48	2	3.00	0.66 - 13.45	1.5×10^{-1}
Any grade anorexia	4	334	46	301	16	2.49	1.45 - 4.30	9.5×10^{-4}
Any grade lymphopenia	2	86	6	48	3	1.08	0.27 - 4.23	9×10^{-1}
Any grade diarrhea	5	517	174	391	26	4.94	3.21 - 7.62	3.8×10^{-13}
Any grade fatigue	4	334	61	301	43	1.18	0.82 - 1.69	3.5×10^{-1}
Any grade hypertension	3	304	112	271	16	6.07	3.69 - 10.00	1.3×10^{-12}
Any grade hypothyroidism	2	254	88	241	17	5.02	3.08 - 8.19	8.9×10^{-11}
Any grade nausea	4	467	98	361	23	3.10	1.93 - 5.00	3.1×10^{-6}
Any grade proteinuria	2	268	89	253	41	2.15	1.55- 2.97	3.6×10^{-6}
Any grade pruritus	2	254	24	241	34	0.70	0.43 - 1.13	1.5×10^{-1}
Any grade rash	4	467	133	361	15	6.50	3.76 - 11.25	2.1×10^{-11}

RR, relative risk; CI, confidence interval; ICT, immune checkpoint inhibitors; VEGFi, vascular endothelial growth factor inhibitor.

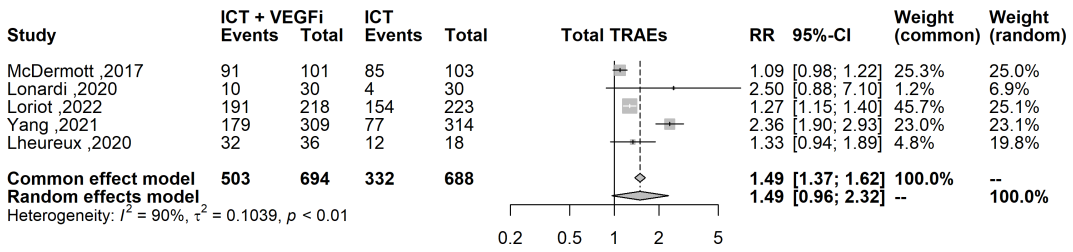

FIGURE 2
Forest plot comparing the risk of any grade TRAEs between ICT vs combination of ICT with VEGFi.

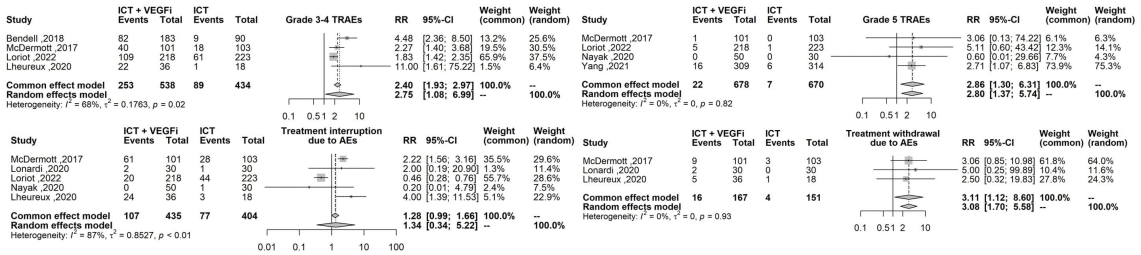

FIGURE 3
Forest plot comparing the risk grade 3-4 TRAEs, grade 5 TRAEs, the risk of treatment interruption and treatment withdrawal between ICT vs combination ICT with VEGFi.

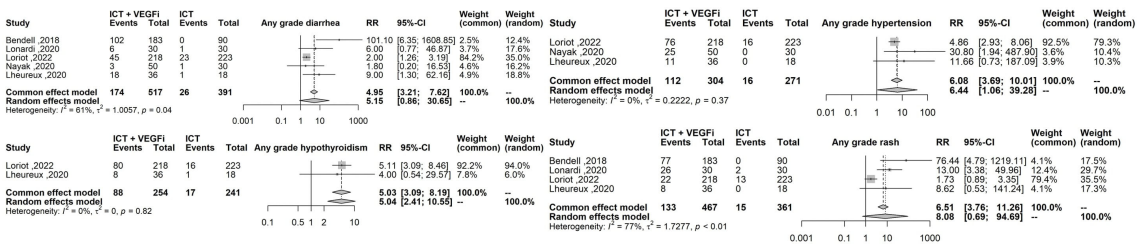

FIGURE 4
Forest plot comparing the risk of the highest effect size TRAEs: sny grade diarrhea any grade hypertension, any grade hyphothyroidism, any grade rash.

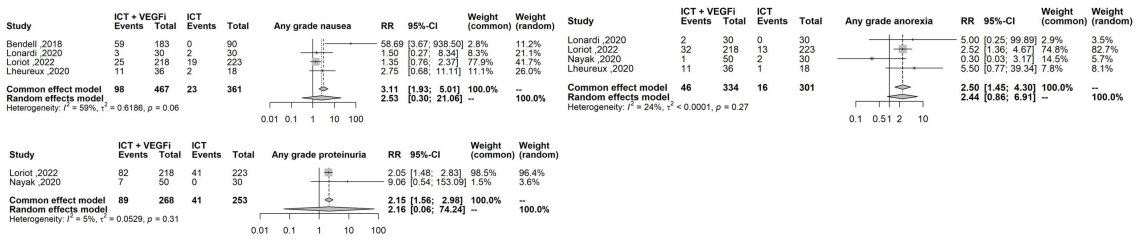

FIGURE 5
Forest plot comparing risk of any grade nausea, any grade proteinuria, any grade anorexia.

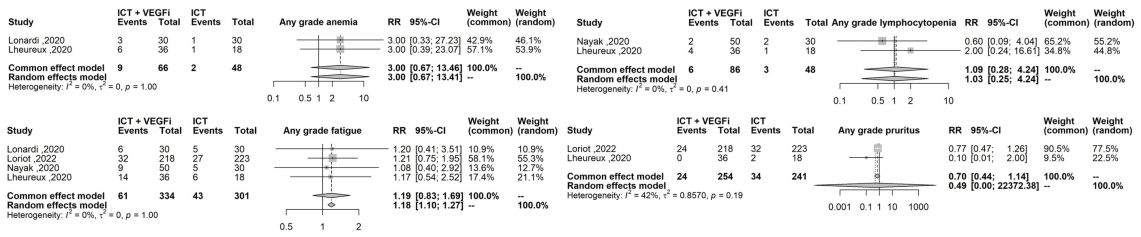

FIGURE 6
Forest plot comparing the risk of any grade anemia, any grade lymphopenia, any grade fatigue, any grade pruritus.

that explored the outcomes of patients with renal cell carcinoma (RCC) treated with cabozantinib combined with nivolumab and ipilimumab versus nivolumab and ipilimumab alone, a stark difference in the occurrence of high-grade adverse events was observed. Specifically, the group treated with the combination therapy experienced grade 3-4 adverse events with greater frequency (79% *vs* 56%). The nearly two-fold increase in ICI-related toxicity in the combination therapy group compared to the monotherapy group in the COSMIC-312 trial is a poignant revelation. Such findings underscore the necessity of understanding the potential synergistic effects on toxicity when combining ICIs with other targeted agents. While combination therapies often seek to exploit complementary mechanisms of action to achieve superior antitumor efficacy, they may also inadvertently amplify the risk of severe adverse events. This amplification in toxicity could result from the simultaneous modulation of multiple pathways, leading to unforeseen interactions that heighten patient risk (27).

To date, three meta-analyses have been conducted to evaluate the safety profile of a combination therapy of ICT and VEGFi. All three studies compared the combination therapy to VEGFi monotherapy and are summarized in Table 4. The study by He et al. noted an increased risk of Grade 3-4 TRAEs, which is consistent with the results of our study. However, there was no analysis performed on the breakdown of AEs (2). The meta-analysis by Tao et al. only evaluated specific AEs that are monitored in RCC, limiting the scope of their results. Nonetheless, they found an increased risk of hypertension, proteinuria, and rash with ICT plus VEGFi combination compared with VEGFi alone (1), which we also noted in our comparison between ICT plus VEGFi versus ICT monotherapy. The meta-analysis by Rizzo et al. aimed to compare the combination of ICT with TKIs to TKI monotherapy in terms of the risk of gastrointestinal toxicity. They noted an increased risk of diarrhea and decreased appetite (referred to as anorexia in our study) in the ICT and TKI combination group compared with TKI monotherapy (3). Because ICT monotherapy is now a standard option across different malignancies, our study provides additional context that can inform clinical decision-making and current practice patterns by comparing the TRAEs with ICT plus VEGFi versus ICT monotherapy. Increased awareness of the specific TRAE risks associated with the combination of ICT with VEGFi will help to monitor, prevent, and treat treatment toxicities in a timely manner (14, 15). However, further data is needed to fully understand the risk of irAEs with ICT plus VEGFi versus ICT monotherapy.

The amalgamation of ICT with VEGFi introduces a complex interplay of enhanced therapeutic potential against the backdrop of augmented toxicities, a challenge particularly evident in kidney cancer. Our meta-analysis, delineating the adverse event profile of ICIs in isolation versus their concomitant administration with VEGFis, underscores this potential enhanced toxicity. Notably, in the realm of kidney cancer, most of trials that combine immunotherapy often employ doses lower than when used in monotherapy. This dose reduction, in part, stems from concerns over enhancing toxicity. Such strategies highlight the importance of an intricate balancing act to maintain clinical sustainability. A potential avenue to sustain treatment efficacy while minimizing adverse effects is to employ reduced drug doses, coupled with individualized therapeutic modulation, informed by early surveillance and predictive biomarkers. By harnessing insights from our meta-analysis, clinicians can judiciously navigate the nexus of potency and safety, optimizing the therapeutic window of these combinatorial regimens.

TABLE 4 Summary of meta-analyses results evaluating the risk of toxicities of ICT and VEGFi combination therapy.

Author	Number of studies included	Treatment	Cancer type	Results (combination therapy vs monotherapy)
He et al.	6	ICT + VEGFi vs VEGFi	RCC	Equal risk of Grade 3-4 TRAEs.
Tao et al.	6	ICT + VEGFi vs VEGFi	RCC	Increased risk of all-grade hypertension, arthralgia, rash, proteinuria, grade 3–5 arthralgia, and proteinuria. Equal risk of grade 3-4 hypertension, grade 3-5 rash. Decreased risk of HFSR, stomatitis, dysgeusia.
Rizzo et al.	4	ICT + TKIs vs TKI	RCC	Increased risk of all-grade diarrhea, grade 3–4 decreased appetite. Decreased risk of all-grade nausea

ICT, immune checkpoint inhibitor therapy; VEGFi, vascular endothelial growth factor inhibitor; TKI, tyrosine kinase inhibitor; HFSR, hand-foot skin reaction.

Our study has several limitations. Only 2 peer-reviewed publications were included, and most studies were available only as abstracts, making it difficult to assess the risk of bias. Additionally, our study assumed that the type of cancer does not impact immunotherapy toxicity, while some studies have suggested that the risk of immunotherapy toxicity may be higher in certain types of cancer, such as lung cancer (28, 29). Our analysis only included one RCT that evaluated the safety profile of ICT versus ICT with VEGFi combination therapy in non-small cell lung cancer (24). Furthermore, we had a limited number of studies, with only one study reporting irAEs, an outcome of interest which we were not able to include in our meta-analysis. The publication of the full manuscript reports of trials is warranted, with particular focus on the risk of irAEs with ICT and VEGFi combination therapy compared with ICT monotherapy. One notable limitation of our study pertains to the absence of a sensitivity analysis that would account for both the diversity of pathologies and the consideration of prior treatments. Our dataset was constrained in its ability to permit such an analysis due to the paucity of subgroup data. Only three studies within our collection provided details on previous treatment lines, a factor known to potentially influence TRAE profiles. Additionally, while it is well-documented in prior research that patients with melanoma and non-small cell lung cancer (NSCLC) exhibit a heightened risk of TRAEs upon sensitivity analysis, our meta-analysis did not encompass patients with melanoma and incorporated data from just one study addressing NSCLC (15). Such omissions and data limitations could curtail the broader applicability and comprehensiveness of our findings in the oncological realm.

Conclusion

We found that ICT plus VEGFi combinations yield an increased risk of specific treatment-related adverse events (TRAEs) compared to ICT alone. Healthcare providers should be aware of the elevated risks for specific TRAEs when using the ICT + VEGFi combination therapy, including rash, hypertension, hypothyroidism, diarrhea, nausea, anorexia, and proteinuria. Further studies are necessary to fully understand the risk of irAEs associated with this combination therapy and provide more granular details on the causes of AEs.

Author contributions

IK: conception; performance of work; interpretation of data; writing the article. WL: performance of work; interpretation of data; writing the article. YG: conception; performance of work. PM: interpretation of data; writing the article. RB: conception; performance of work; interpretation of data; writing the article. OA: conception; performance of work; interpretation of data; writing the article. PG: interpretation of data; drafting the work; provided final approval of the version to be published. 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2023.1238517/full#supplementary-material

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Current vaccination status and safety of children with peripheral neuroblastoma in the real-world

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Background: peripheral neuroblastic tumors (pNT) have high incidence and mortality, and infants are prone to various infectious diseases. The purpose of this study is to understand the immunization status of children with pNT in the real-world and the incidence of adverse reactions after vaccination, and to evaluate the feasibility of vaccination and the influencing factors of vaccination.

Methods: Children with pNT treated in the Children's Hospital Affiliated to Zhejiang University from January 1, 2011 to December 1, 2021 were included. By referring to medical records, the vaccination history of the national immunization program (NIP) vaccines and the occurrence of adverse events following immunization(AEFI), current status and safety of immunization in children with pNT in the real-world were analyzed.

Results: Among 784 children with pNT, 394 were able to obtain the history of vaccination. The overall vaccination rate of NIP vaccines was 71.49% before chemotherapy and 37.67% after chemotherapy, and the recovery time of vaccination after treatment was 16.00 (6.00,24.00) months. Age, time of tumor diagnosis and disease classification were significantly correlated with vaccination. AEFI reported an incidence of 0.23‰.

Conclusion: The vaccination rate of children with pNT is generally low, especially the vaccination rate after chemotherapy. The vaccination safety is good, children should be encouraged to immunize.

KEYWORDS

peripheral neuroblastoma, children, immunization rates, security, immunization program

1 Introduction

Cancer is the second leading cause of death among children aged 5 to 14 years after accidents, and more than one third of children diagnosed with tumors are before the age of five (1). In 2020, a retrospective survey of 938 children in Beijing over the past decade found that the three most common types of infant malignant solid tumors were retinoblastoma (39%), neuroblastoma (28.4%) and hepatoblastoma (14.2%) (2). Neuroblastoma is one of peripheral neuroblastic tumors (pNT). The International Neuroblastoma Pathology Classification has developed the classification of pNT (3), including 4 types of tumors: 1) neuroblastoma (NB); 2) ganglio-neuroblastomainter mixed (GNBi); 3) ganglioneuroma (GN); 4) ganglio-neuroblastomanodular (GNBn), the first 3 types representing the maturation process of NB, they are rare benign tumors (4). The last type is polyclonal, the differentiation degree of GNB is between NB and GN, and the malignancy degree is lower than NB (5).

All ganglioneuromas used to be NB in early development, and ganglioneuromas are rare in infancy compared to older children, so NB is the most common and deadly tumor in pNT (6). If children were diagnosed to be in the late stage of NB, the prognosis is poor (7). It accounts for 8-10% of all childhood cancer cases, with the highest incidence occurring in newborns under one year of age, 80.8% in children under five years of age and only 1.6% in children over 10 years of age, with more males than females (8). In the United States, the incidence of NB is 11-13 cases per million children under 15 years of age (9); In China, the incidence of NB among live births is about 7.7 cases per million (10).

Children with tumor have great damage to their immune ability due to the disease itself and chemotherapy (11–13). Studies have shown that the prognosis of high-risk children is much worse than that of low-risk and medium-risk children, and the poor prognosis is closely related to low immune capacity (14 15).This is because high-risk neuroblastoma tumors are characterized by a low number of immune cells in the tumor microenvironment and are commonly referred to as “cold” tumors (14). Patients with neuroblastoma typically present with an impaired immune system at the time of diagnosis, and while chemotherapy can lead to an initial increase in total white blood cell and lymphocyte counts (15), further aggressive treatment can suppress immune function (16). Consequently, it can result in the disruption of both humoral and cellular immunity in children with neuroblastoma (17). In terms of humoral immunity, there is a decrease in levels of immunoglobulins IgG, IgA, IgM, and IgE. Regarding cellular immunity, there is a reduction in total T lymphocytes (TTL) as well as in the CD4+/CD8+ ratio compared to levels prior to chemotherapy (18). After therapy in the CD8 T cell compartment, signs of immune reconstitution were observed five years after diagnosis and treatment (19). However, no immune reconstitution was observed with autologous hematopoietic stem cell transplantation and increased immunotherapy (20). Therefore, the child’s immune system is weak after diagnosis and treatment, which can lead to a high rate of infection (21, 22). Study have found that NB patients have a high burden of infection, with a cumulative incidence of 45% during treatment, and an infection rate (IR) of 0.19/100 patient-days-at risk (23).

In recent years, the vaccination coverage rate of children under the National Immunization Program (NIP) in China has reached over 90% (24), the vaccination rate of children under the NIP is good. However, a survey of children receiving medical treatment in Guangzhou showed that 87.8% of them were delayed due to special diseases (25), the vaccination rate among children with tumors and other malignant diseases was significantly lower than that of normal children (26, 27), the completion rate of planned immunization is low.

Immunization, an essential approach to bolster immune function and reduce infections, also plays a significant role in enhancing the prognosis of immune levels in children with tumors (28, 29). Research has demonstrated a low occurrence of adverse reactions among vaccinated children (30), including those diagnosed with neuroblastoma (31, 32). By collecting and sorting disease data and immunization data of pNT children after chemotherapy, this study analyzed the current immunization status of children and the occurrence of adverse reactions by using the analysis method of status study, and obtained the immunization coverage rate and incidence of adverse reactions of pNT children, providing suggestions and guidance for pNT children immunization.

2 Materials and methods

2.1 Study design

A retrospective study was conducted on children diagnosed as pNT and treated in Children’s Hospital of Zhejiang University from January 1, 2011 to December 1, 2021.

2.2 Participants

A total of 784 children who had been diagnosed with peripheral neuroblastoma by oncologists and who had received treatment were included. Exclusion criteria included patients with other systemic malignancies and patients over 16 years of age at first diagnosis. The pNT classification includes four types of tumors: 1) NB; 2) GNBi; 3) GN; 4) GNBn. The children in the study were categorized as either belonging to Hangzhou or non-Hangzhou. Additionally, the age of the children was grouped into four categories: 1-5 years, 5-10 years, 10-15 years, and 15+ years, with each group separated by a five-year interval. The tumor diagnosis age was the child’s first admission to the hospital for chemotherapy, while whose actual age was determined from their year of birth to July 2022. Lastly, the vaccination recovery time refers to the duration between the first vaccination after completing chemotherapy.

2.3 Data collection

The patient's clinical and basic information is collected from the hospital's tumor reporting system, including the patient's name, sex, age, household registration, date of hospitalization, hospitalization number, disease diagnosis and treatment history. The vaccination records of target participants were extracted from the Zhejiang Provincial immune Information System. Data on Abnormal Reactions to Vaccination (AEFI) were collected from the China National Adverse Event Information System for Immunization.

In this study, the Immunization records collected included 13 types of NIP vaccines with 28 doses each (Table 1). Replacement vaccinations are also equivalent to vaccination completion.

Adverse events following immunization (AEFI),including: adverse reactions (including general reactions and abnormal reactions), vaccine quality incidents, vaccination incidents, coincidences, and psychogenic reactions.

2.4 Correlation index calculation

$$\text{Vaccination coverage}=\frac{\text{The number of people vaccinated in a study population over a given period of time}}{\text{The population of the study population at the same time}}\times 100\,\%$$

$$\text{Incidence of adverse reactions}=\frac{\text{The number of adverse reactions occurring in the vaccinated population over a given period of time}}{\text{Total number of vaccination doses in the same time period}}\times 100\,\%$$

2.5 Statistical analysis

Basic Excel software was used for data screening and sorting, and then SPSS 25.0 software was used for data analysis. Normal measurement data were represented by (X ± S), T-test was used for comparison between two groups, and analysis of variance was used for comparison between multiple groups. Non-normal measurement data were represented by M (P_{25},P_{75}), and rank sum test was used for comparison between groups. The rate or percentage of counting data was expressed, and the chi-square test was used for comparison between groups. Logistic regression model was used to analyze the influencing factors. $P<0.05$ was considered statistically significant.

In the analysis, according to the age distribution, the actual age was analyzed as a group of 5 years, while the tumor diagnosis age was analyzed as a group of 36 months, for the median age at tumor diagnosis was 34 months (7).

2.6 Ethical considerations

This project was approved by the Ethics Committee of Hangzhou Center for Disease Control and Prevention (judgment reference number: 2021-18) and the Ethics Committee of the School of Medicine, Children's Hospital of Zhejiang University (judgment number: 2022-IRB-028).

3 Results

3.1 Demographic characteristics

A total of 394 children from 784 were given vaccination data. According to the inoculation data, 52.79% (208/394) were males and 47.21% (186/394) were females. The local population accounted for 31.22% (123/394) and the non-local population accounted for 68.78% (271/394). 53.81% of the patients were 5-10 years old; 45.43% of the patients were younger than 36 months of age; 81.98% of the cases were NB, 16.50% GNBi and 1.52% GN (Table 2).

3.2 Vaccination status of NIP vaccines

The coverage rates of the first, second and third doses of HepB vaccine were 95.18%, 93.40% and 78.93% before chemotherapy, and were 10.53%, 23.08% and 54.22% after chemotherapy; the coverage rate of BCG vaccine was 91.62% before chemotherapy, and was 21.21% after chemotherapy, the coverage rates of the first and second doses of IPV vaccine were 91.37% and 88.07% before chemotherapy, and were 38.24% and 38.30% after chemotherapy; the coverage rates of the first and second doses of bOPV vaccine were 83.76% and 46.51% before chemotherapy, and were 46.88% and 27.54% after chemotherapy. The vaccination rates of the first, second, third and fourth doses of DTaP vaccine were 87.82%, 86.29%, 84.77% and 66.49% before chemotherapy, and were 41.67%, 44.44%, 41.67% and 42.97% after chemotherapy. The vaccination rates of DT vaccine was 21.05% before chemotherapy, and was27.56% after chemotherapy; and the vaccination rates of the first and second doses of MMR vaccine were 87.56% and 77.16% before chemotherapy, and were 46.88% and 27.54% after chemotherapy. The vaccination rates of the first and second doses of JE-L vaccine were 72.59% and 60.31% before chemotherapy, and were 50.00% and 36.59% after chemotherapy; the vaccination rates of the first, second, third and fourth doses of JE-I vaccine were 74.37%, 71.32%, 46.31% and 16.14% before chemotherapy, and were 48.51%, 53.10%, 54.37% and 12.50% after chemotherapy; and the vaccination rates of the first and second doses of MPSV-A vaccine were 77.41% and 69.90% before chemotherapy, and were 33.71% and 29.41% after chemotherapy. The coverage rates of the first and second doses of MPSV-AC vaccine were 77.75% and 17.89% before chemotherapy, and were 29.41% and 63.64% after chemotherapy. HepA-L vaccine was 63.61% before chemotherapy, and was 43.88% after chemotherapy; and the first and second doses of HepA-I vaccine 66.75% and 63.35%before chemotherapy, and were 7.09% and 37.86% after chemotherapy. The total vaccination rate was 71.49% before chemotherapy, and was 37.67% after chemotherapy. Except that there were no difference in the vaccination rate before and after chemotherapy for DT vaccine, the third and fourth doses of JE-I vaccine, and the vaccination rate after chemotherapy for the second dose of MPSV-AC was higher, the vaccination rate after chemotherapy for other NIP vaccines were significantly lower than that before chemotherapy (Table 3).

TABLE 1 National Immunization Program Vaccine Immunization Schedule for Children (2021 version).

Vaccines	Recommended age for vaccination													
	at birth	1 month	2 months	3 months	4 months	5 months	6 months	8 months	9 months	18 months	2 years	3 years	4 years	6 years
HepB	1st dose	2nd dose					3rd dose							
BCG	1st dose													
IPV			1st dose	2nd dose										
bOPV					1st dose								2nd dose	
DTaP				1st dose	2nd dose	3rd dose				4th dose				
DT														one dose
MMR								1st dose		2nd dose				
JE-L								1st dose			2nd dose			
JE-I								1st and 2nd dose			3rd dose			4th dose
MPSV-A							1st dose		2nd dose					
MPSV-AC												1st dose		2nd dose
HepA-L										one dose				
HepA-I										1st dose	2nd dose			

Note: 1. When the live attenuated JE vaccine was selected, two doses of vaccination should be used. When the inactivated JE vaccine was selected, four doses of vaccination were used; The interval between the first and second doses of JE vaccine was 7-10 days.
2. When choosing live attenuated hepatitis A vaccine for vaccination, a one-dose vaccination procedure is used. When selecting the inactivated hepatitis A vaccine, two doses of vaccination are used.
HepB, Hepatitis B vaccine; BCG, Bacillus Calmett-Guerin vaccine; IPV, Inactivated polio vaccine; bOPV, Oral live attenuated polio vaccine; DTaP, Acellular pertussis diphtheria tetanus vaccine; DT, Diphtheria tetanus vaccine; MMR, Measles; mumps, rubella vaccine; JE-L, Live attenuated Japanese encephalitis vaccine; JE-I, Inactivated Japanese encephalitis vaccine; MPSV-A, Meningococcal meningitis-A vaccine; MPSV-AC, Meningococcal meningitis-AC vaccine; HepA-L, Live attenuated Hepatitis A vaccine; HepA-I, Inactivated Hepatitis A vaccine.

10.3389/fimmu.2023.1278258

TABLE 2 Sample characteristics (N=394).

Characteristics	N	%
Gender		
Male	208	52.79
Female	186	47.21
Location		
Hangzhou	123	31.22
non-Hangzhou	271	68.78
Actual age (year)		
1-5	109	27.66
6-10	212	53.81
11-15	62	15.74
16-	11	2.79
Tumor diagnosis age (month)		
0-36	179	45.43
37-72	147	37.31
73-108	39	9.90
109-	25	6.35
Pathological classification		
NB	323	81.98
GNBi	65	16.50
GNBn	0	0.00
GN	6	1.52
Total	394	100

NB, neuroblastoma; GNBi, ganglio-neuroblastomainter mixed; GN, ganglioneuroma; GNBn, ganglio-neuroblastomanodular.

3.3 The status of re-vaccination after diagnosis and treatment

Among the 394 children, 40.60% (160/394) recovered the immunization of NIP vaccine after chemotherapy, accounting for 52.50% (84/160) in males, 47.50%(76/160) in females and 26.87% (43/160) in Hangzhou, Non-Hangzhou accounted for 73.12% (117/160), the median age of diagnosis was 15.00 (4.30,40.00) months, and the median age of resumption of immunization was 16.0 (6.00,24.00) months (Table 4).

3.4 Univariate analysis of factors of immunization status

The basic information and immunization status of children with available vaccination data were included for univariate analysis. For NIP vaccine, there were no statistically significant differences in gender and location ($P > 0.05$), but there were statistically significant differences in age interval and tumor diagnosis time ($P< 0.05$). For the first dose of HepB vaccine(χ^2 = 7.44, P =0.027), the second and fourth doses of DTaP vaccine(χ^2 = 6.16, P =0.042), the second dose of MPSV-AC vaccine (χ^2 = 11.17, P = 0.003), HepA-L(χ^2 = 6.41, P =0.031) and HepA-I vaccine(χ^2 = 5.90, P=0.039), the differences in Disease Classification were statistically significant (Supplementary Tables 1-13).

3.5 Multivariate analysis of factors of immunization status

The basic information and immunization status of children with available vaccination data were included to construct a multi-factor ordered logistics regression equation. The results showed that among 394 children with available vaccination data, the vaccination rate of NIP vaccine was lower with the age of older children than those who were less than 5 years old. Compared with the time of diagnosis less than 36 months, the later the diagnosis time, the higher the vaccination rate. For the second dose of MPSV-AC vaccine, the vaccination rate of NB children with high malignant degree was lower than that of GN children with mild disease (Tables 5, 6).

3.6 Abnormal response to vaccination (AEFI)

According to AEFI monitoring and management information system, there were 2 cases of adverse reactions among NIP vaccines, both occurred after diagnosis and treatment, one case occurred in the third dose of DTaP vaccine, which was redness and swelling at the vaccination site 7 hours after vaccination, the other case occurred in the fourth dose of DTaP vaccine with redness and swelling at the site 4 days after inoculation. The incidence of adverse reactions was calculated was 0.23‰(2/8728). Moreover, complications, severe AEFI, and community AEFI did not occur among these patients.

4 Discussion

In this study, NB was the main tumor type of pNT children, and its basic age, gender and disease diagnosis time were similar to those in domestic and foreign studies (7). In our study, the vaccination data of some children could not be queried, we found that the vaccination coverage rate of NIP vaccines reached 71.49% before chemotherapy and 37.67% after chemotherapy, which was lower than that of normal children in China (24), especially the vaccination rate after chemotherapy. We also see a gradual decline in vaccination rates for different doses of the same vaccine, and the delay has been found in other studies (33). By analyzing the factors related to vaccination, the result show that the age of children, the time of tumor diagnosis and disease

10.3389/fimmu.2023.1278258

TABLE 3 Rate of NIP vaccination in children with pNT.

NIP vaccines	NA-b	NS-b	Vaccination rate (%)	NA-a	NS-a	Vaccination rate(%)	χ^2	*P*
$HepB_1$	375	394	95.18	2	19	10.53	152.77	<0.001
$HepB_2$	368	394	93.40	6	26	23.08	147.02	<0.001
$HepB_3$	311	394	78.93	45	83	54.22	22.12	<0.001
BCG	361	394	91.62	7	33	21.21	120.93	<0.001
IPV_1	360	394	91.37	13	34	38.24	74.23	<0.001
IPV_2	347	394	88.07	18	47	38.30	72.93	<0.001
$bOPV_1$	330	394	83.76	30	64	46.88	40.75	<0.001
$bOPV_2$	60	129	46.51	19	69	27.54	6.71	0.009
$DTaP_1$	346	394	87.82	20	48	41.67	64.01	<0.001
$DTaP_2$	340	394	86.29	24	54	44.44	54.59	<0.001
$DTaP_3$	334	394	84.77	25	60	41.67	58.47	<0.001
$DTaP_4$	254	382	66.49	55	128	42.97	22.21	<0.001
DT	60	285	21.05	62	225	27.56	2.93	0.087
MMR_1	315	394	79.95	30	79	37.97	58.74	<0.001
MMR_2	256	382	67.02	47	126	37.30	34.75	<0.001
$JE\text{-}L_1$	286	394	72.59	54	108	50.00	19.78	<0.001
$JE\text{-}L_2$	237	393	60.31	45	123	36.59	21.26	<0.001
$JE\text{-}I_1$	293	394	74.37	49	101	48.51	25.16	<0.001
$JE\text{-}I_2$	281	394	71.32	60	113	53.10	13.24	<0.001
$JE\text{-}I_3$	182	393	46.31	56	103	54.37	2.12	0.145
$JE\text{-}I_4$	46	285	16.14	2	16	12.50	0.15	0.699
$MPSV\text{-}A_1$	305	394	77.41	30	89	33.71	65.24	<0.001
$MPSV\text{-}A_2$	267	382	69.90	37	115	32.17	52.95	<0.001
$MPSV\text{-}AC_1$	297	382	77.75	25	85	29.41	75.88	<0.001
$MPSV\text{-}AC_2$	51	285	17.89	7	11	63.64	14.06	<0.001
HepA-L	243	382	63.61	61	139	43.88	16.32	<0.001
$HepA\text{-}I_1$	255	382	66.75	9	127	7.09	135.92	<0.001
$HepA\text{-}I_2$	242	382	63.35	53	140	37.86	27.09	<0.001
Total	7402	10354	71.49	891	2365	37.67	970.25	<0.001

NA-b: the number of children who have actually received the vaccine before chemotherapy; NS-b: the number of children who are compulsory to receive the vaccine before chemotherapy. NA-a: the number of children who have actually received the vaccine after chemotherapy; NS-a: the number of children who are compulsory to receive the vaccine after chemotherapy.
HepB, hepatitis B vaccine; BCG, Bacillus Calmette Guerinvaccine vaccine; IPV, inactivated poliomyelitis vaccine; bOPV, Live Attenuated Oral Poliomyelitis Vaccine; DTaP, Diphtheria and tetanustoxoid with acellular pertussis vaccine; DT, Diphtheria Tetanus vaccine; MMR, Measles mumps and rubella vaccine; JE-L, Live attenuated Japanese encephalitis vaccine; JE-I, Inactivated Japanese Encephalitis vaccine; MPSV-A, Meningococcal polysaccharide vaccineA; MPSV-AC, Meningococcal polysaccharide vaccineAC; HepA-L, live attenuated hepatitis A vaccine; HepA-I, Inactivated HepatitisA vaccine.
Replacement vaccinations are also equivalent to vaccination completion.

classification were significantly correlated with whether or not children received some of the vaccines.

In our study, we found that before diagnosis, the vaccination rate of pNT children vaccine was not high, which did not meet the requirements of our country (34). Besides, the vaccination rate of booster immunization (multiple vaccinations) of all vaccines was lower than that of basic immunization (primary vaccinations), which was consistent with the findings of CAO related the vaccination rate of Chinese children in 2012 (35). Importantly, the vaccination rate of these patients >7 years old was lower than 80%.The possible reason were that: a) national vaccination requirements in China before 2006 was low; b) many vaccines

TABLE 4 Diagnosis and recovery of the NIP vaccine after treatment.

characteristics	NIP
	N(%)
Gender	
Male	84(52.50)
Female	76(47.50)
Location	
Hangzhou	43(26.87)
non-Hangzhou	117(73.12)
Tumor diagnosis age (month) *	15.00(4.30,40.00)
Resumption of immunization time (month)*	16.00(6.00,24.00)
Total	160(40.60)

*The expression is M (P25,P75).

were not included in our National Immunization Program in early years; c) vaccination records were handwritten and lacked a unified electronic information management system before 2006, which may lead to the loss of some information (36). For the reasons mentioned above, patient who were born before 2006 had a lower vaccination rate.

We found that the older the child, the lower the vaccination rate; the earlier the tumor diagnosis, the lower the vaccination rate; the more severe the disease classification of children, the lower the coverage rate of the second dose of MPSV-AC vaccine, and they are consistent with the current survey results (37, 38). The possible reasons are as follows: Firstly, it is related to the limitation of vaccination age. The recommended age for NIP vaccination is less than three years old in most cases and less than six years old at the latest; Secondly, it is related to the diagnosis of the disease. Parents focus on the treatment of the disease, and their compliance to immunization is reduced. Moreover, due to the lack of medical knowledge, they have certain concerns about the safety of immunization (39); Thirdly, doctors may recommend stopping vaccination after diagnosis because of the risk associated with vaccination (33); Fourthly, according to the immunization strategy for special children (40), live attenuated vaccines are prohibited for children within three months of chemotherapy, so the immunization plan of children is easy to be delayed.

In our study, it is found that 40.60% (160/394) of the patients were restored to immunization with NIP vaccine after treatment, and the median age of restored immunization was 16.00 (6.00,24.00) months. Only 2 cases of AEFI reaction were found, accounting for 0.23‰. Compared with normal children, the incidence of AEFI reaction was similar (41). In addition, a large number of studies have shown that immunization of pNT children is safe and feasible (28, 31, 42), so a series of measures can be taken to restore the immunization of children. Special services have been set up in recent years to provide vaccination advice for children with special needs: A multidisciplinary immunization model was established for children with hematologic tumors in China, and the incidence of AEFI was 5.9% (43), they also revised the guidelines for the children with cancer, including the type, timing, dosage and even immunization procedures (44).

Because of the effect of disease and treatment, the level of autoantibody and immunity of pNT children is greatly reduced (45). And the 5-year survival rate of high-risk children with NB is less than 2%, but the 5-year survival rate of low- and intermediate-risk children reaches 75%-98%; therefore, children with this disease can be effectively prolonged by improving their prognosis (46). Since immune capacity maybe related to prognosis, while the

TABLE 5 Multivariate analysis of different age groups.

NIP vaccines	Age(year)						
	1-5*	6-10		11-15		16-	
		OR(95%CI)	*P*	OR(95%CI)	*P*	OR(95%CI)	*P*
$HepB_1$	1	0.82(0.07,9.59)	0.006	0.16(0.01,2.17)	0.000	0.01(0.00,0.26)	0.004
$HepB_2$	1	1.00(0.21,4.73)	0.992	0.27(0.04,1.71)	0.166	0.02(0.00,0.19)	0.001
$HepB_3$	1	2.01(0.88,5.00)	0.095	0.66(0.19,2.25)	0.507	0.13(0.02,0.93)	0.043
BCG	1	1.09(0.36,3.35)	0.876	0.35(0.08,1.49)	0.155	0.04(0.00,0.31)	0.002
IPV_1	1	1.39(0.37,5.19)	0.623	0.33(0.07,1.76)	0.197	0.02(0.00,0.21)	0.001
IPV_2	1	1.41(0.49,4.00)	0.516	0.59(0.14,2.47)	0.460	0.04(0.00,0.35)	0.004
$bOPV_1$	1	1.82(0.45,7.35)	0.399	0.39(0.07,2.09)	0.272	0.02(0.00,0.25)	0.002
$bOPV_2$	1	1.65(0.57,4.86)	0.357	0.63(0.15,2.76)	0.544	0.04(0.00,0.39)	0.005
$DTaP_1$	1	1.13(0.36,3.49)	0.835	0.65(0.14,3.05)	0.587	0.05(0.00,0.45)	0.008
$DTaP_2$	1	1.37(0.49,3.82)	0.553	0.74(0.17,3.22)	0.688	0.06(0.00,0.54)	0.011
$DTaP_3$	1	1.72(0.74,3.98)	0.206	1.09(0.28,4.27)	0.894	0.07(0.00,0.55)	0.011
$DTaP_4$	1	1.60(0.91,2.83)	0.104	1.76(0.57,5.47)	0.323	0.14(0.02,0.92)	0.041

(Continued)

TABLE 5 Continued

NIP vaccines	Age(year)						
	1-5*	6-10		11-15		16-	
		OR(95%CI)	*P*	OR(95%CI)	*P*	OR(95%CI)	*P*
MMR_1	1	3.03(1.43,6.44)	0.004	1.18(0.35,3.94)	0.786	0.19(0.02,1.61)	0.130
MMR_2	1	2.43(1.37,4.30)	0.002	1.82(0.60,5.48)	0.290	0.10(0.02,0.69)	0.020
JE-L_1	1	2.14(1.05,4.35)	0.036	1.31(0.38,4.53)	0.670	0.09(0.01,0.72)	0.023
JE-L_2	1	2.38(1.39,4.05)	0.001	1.99(0.73,5.41)	0.176	0.18(0.03,1.16)	0.072
JE-I_1	1	2.17(1.04,4.49)	0.038	1.27(0.36,4.46)	0.701	0.08(0.11,0.67)	0.018
JE-I_2	1	2.29(1.11,4.71)	0.025	1.35(0.38,4.67)	0.640	0.09(0.01,0.69)	0.021
JE-I_3	1	2.17(1.27,3.70)	0.004	1.76(0.65,4.84)	0.267	0.16(0.03,0.99)	0.049
MPSV-A_1	1	1.37(0.69,2.72)	0.373	0.88(0.29,2.74)	0.831	0.05(0.01,0.33)	0.002
MPSV-AC_1	1	1.67(0.98,2.83)	0.060	1.88(0.73,4.93)	0.194	0.12(0.02,0.75)	0.024
HepA-L	1	1.61(0.91,284)	0.103	0.88(0.31,2.48)	0.811	0.02(0.00,0.17)	0.000
HepA-I_1	1	1.65(0.93,2.91)	0.088	0.84(0.29,2.38)	0.736	0.02(0.00,0.17)	0.000
HepA-I_2	1	1.76(1.02,3.07)	0.044	0.69(0.26,1.86)	0.461	0.03(0.00,0.20)	0.000

HepB, Hepatitis B vaccine; BCG, Bacillus Calmett-Guerin vaccine; IPV, Inactivated polio vaccine; bOPV, Oral live attenuated polio vaccine; DTaP, Acellular pertussis diphtheria tetanus vaccine; DT, Diphtheria tetanus vaccine; MMR, Measles, mumps, rubella vaccine; JE-L, Live attenuated Japanese encephalitis vaccine; JE-I, Inactivated Japanese encephalitis vaccine; MPSV-A, Meningococcal meningitis-A vaccine; MPSV-AC, Meningococcal meningitis-AC vaccine; HepA-L, Live attenuated Hepatitis A vaccine; HepA-I, Inactivated Hepatitis A vaccine.
*Reference group: according to the age distribution, the actual age was analyzed as a group of 5 years.

damage/alteration to the immune system caused by the pNT. This also reminds us it may also be helpful to distinguish the categorization of pNT patients into high-risk, medium-risk, and low-risk categories as it relates to immune status (15, 16). So parents, doctors should take a correct view of the condition (36, 47) that children with tumors can be immunized, and we can take actions to improve the level of children's autoimmune, and children's prognosis and quality of life.

The key advantage of this study is that there are few studies on immunization coverage in children with pNT. With the increasing

TABLE 6 Multivariate analysis of different tumor diagnosis time.

NIP vaccines	tumor diagnosis age(month)						
	0-36*	37-72		73-108		109-	
		OR(95%CI)	*P*	OR(95%CI)	*P*	OR(95%CI)	*P*
$DTaP_4$	1	4.15(1.86,9.26)	0.001	3.47(0.95,12.64)	0.060	1.71(0.34,8.72)	0.518
DT	1	1.03(0.55,1.88)	0.933	16.88(5.23,54.56)	0.000	10.69(2.16,52.76)	0.004
MMR_2	1	5.26(2.24,12.34)	0.000	4.82(1.21,19.27)	0.026	3.97(0.69,22.91)	0.123
JE-L_2	1	4.64(2.31,9.28)	0.000	8.22(2.03,33.24)	0.003	4.33(0.86,2.94)	0.077
JE-I_3	1	5.28(2.59,10.76)	0.000	9.36(2.29,38.33)	0.002	4.94(0.97,25.08)	0.054
JE-I_4	1	4.76(2.51,9.04)	0.000	7.36(2.21,24.53)	0.000	3.01(0.69,13.16)	0.143
MPSV-A_2	1	3.67(1.69,7.93)	0.001	4.28(1.12,16.31)	0.033	2.94(0.48,18.07)	0.244
MPSV-AC_1	1	6.48(3.45,12.20)	0.000	14.29(3.94,51.76)	0.000	13.24(2.32,75.29)	0.004
MPSV-AC_2	1	1.19(0.63,2.27)	0.595	18.02(5.94,54.62)	0.000	9.43(2.01,44,17)	0.004
HepA-L	1	3.96(1.83,8.55)	0.000	12.52(2.12,73.83)	0.005	10.33(1.31,81.67)	0.027
HepA-I_1	1	4.33(1.96,9.69)	0.000	12.46(2.11,73.73)	0.005	10.59(1.33,84.49)	0.026
HepA-I_2	1	4.73(2.21,10.07)	0.000	14.55(2.77,76.43)	0.000	7.74(1.38,43.49)	0.020

DTaP, Acellular pertussis diphtheria tetanus vaccine; DT, Diphtheria tetanus vaccine; MMR, Measles, mumps, rubella vaccine; JE-L, Live attenuated Japanese encephalitis vaccine; JE-I, Inactivated Japanese encephalitis vaccine; MPSV-A, Meningococcal meningitis-A vaccine; MPSV-AC, Meningococcal meningitis-AC vaccine; HepA-L, Live attenuated Hepatitis A vaccine; HepA-I, Inactivated Hepatitis A vaccine.
*Reference group: The tumor diagnosis age was analyzed as a group of 36 months, for the median age at tumor diagnosis was 34 months.

awareness of childhood immunization against diseases, more studies are needed to provide valuable data for the relevant departments' immunization programs. The limitation of this study is that the data of the objects of this study were from the Children's Hospital Affiliated to Zhejiang University, which was not randomly selected and could not represent all the children in the whole population, and there was selection error. In addition, the data of this study came from a retrospective study in the real world, which was an observational study and the level of evidence was not strong enough.

5 Conclusion

The vaccination coverage rate of pNT children is low, especially the vaccination rate after chemotherapy, and the incidence of AEFI is also low. Therefore, in-depth research on the vaccination of children should be carried out. Parents, doctors, the society and the government should also strengthen the awareness of the safety of immunization, orderly guide the immunization of children, and reduce infection.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This project was approved by the Ethics Committee of Hangzhou Center for Disease Control and Prevention (reference number: 2021-18) and the Ethics Committee of the School of Medicine, Children's Hospital of Zhejiang University (reference number: 2022-IRB-028).

Author contributions

HS: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. YX: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Writing – original draft, Writing – review & editing. YZ: Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. YL: Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. XZ: Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing. ML: Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. CJ: Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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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.2023.1278258/full#supplementary-material

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