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Disputes and challenges of immune checkpoint inhibitors in gastrointestinal cancers

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Disputes and challenges of immune checkpoint inhibitors in gastrointestinal cancers

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Long survival in a pancreatic carcinoma patient with multi-organ toxicities after sintilimab treatment: A case report

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Pancreatic carcinoma is the leading cause of death among digestive malignancies in China. In particular, there is no breakthrough in prolonging the survival of pancreatic cancer patients with chemical and targeted therapies. Tumor immunotherapy brings opportunities and progress for the treatment of pancreatic cancer. Sintilimab is an innovative PD-1 inhibitor which was reported certain clinical benefits in multi-line treatments of advanced pancreatic cancer with gemcitabine. The combination therapy of PD-1 with gemcitabine plus high-intensity focused ultrasound (HIFU) in pancreatic cancer has not been reported. Here we report a case of a Chinese old patient diagnosed with metastatic pancreatic cancer. Two months after sintilimab treatment, the patient occurred severe immune colitis. The patient was diagnosed with immune ureteritis after 8 months of treatment. The immue-related adverse events (irAEs) refined after timely recognition and correct intervention by the clinician and clinical pharmacist. After first-line treatment of sintilimab plus gemcitabine combined with pancreatic HIFU, the patient achieved a remarkable benefit of 11-month progression-free survival (PFS) and 20-month overall survival (OS). The first-line treatment of sintilimab plus gemcitabine combined with HIFU demonstrates a potential therapeutic effect on metastatic pancreatic carcinoma with tolerable adverse reactions.

KEYWORDS

PD-1, pancreatic carcinoma, immune-related adverse events, long survival, case report

1 Introduction

Pancreatic carcinoma is a malignant tumor of digestive system with a very high degree of malignancy and poor prognosis. In China, the incidence and mortality of pancreatic cancer are annually increasing, and the mortality rate is close to the incidence (Cao et al., 2021). Pancreatic cancer is characterized by strong occult, aggression, easy to metastasize, and tolerant to chemoradiotherapy. The 5-year survival rate of pancreatic cancer is very low (less than 8%), and the median survival time is only 6 months (McGuigan et al., 2018). The main methods of treatment for pancreatic cancer include surgery, radiotherapy, chemotherapy and interventional therapy. Many clinical studies have shown the advantage of HIFU in pain reduction, extension of survival time, improvement of performance status and the great safety in advanced pancreatic patients, which confirmed it as a promising modality for palliative therapy (Xiaoping and Leizhen, 2013; Zhou, 2014; Marinova et al., 2016). It has been approved

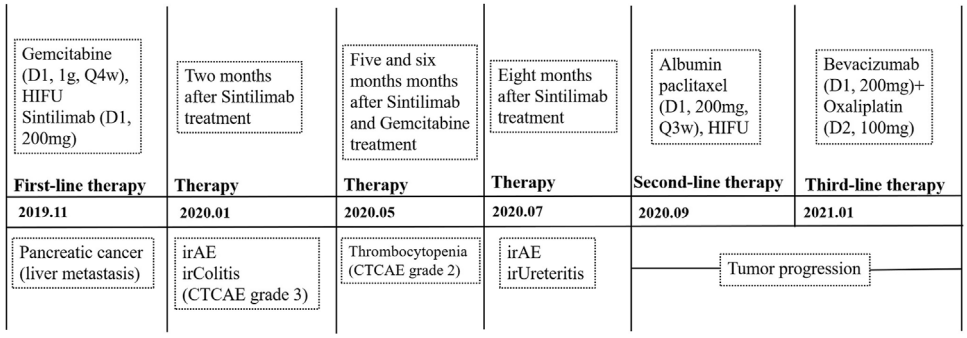


FIGURE 1
Schematic timeline of PD-1 treatment and its immune-related adverse events.

by the China Food and Drug Administration (CFDA) for the treatment of metastatic pancreatic cancer.

Therapy that targets programmed death-1 or programmed death-ligand 1 (PD-1/PD-L1), which are known as immune checkpoints, has been rapidly developing as oncotherapy for various carcinomas recently. PD-1 inhibitors restore endogenous antitumor T Cell responses by blocking the interaction of PD-1 with its ligand PD-L1 (Han et al., 2020). The development of tumor immunotherapy has been brought opportunities and progress to the treatment of pancreatic cancer (Mucileanu et al., 2021). Although PD-1 inhibitors have produced impressive results on varied cancers, they also caused a series of immune-related adverse events (irAEs), which often involve the skin, intestine, liver, lung, endocrine and other target organs (Darnell et al., 2020). Given the immune-mediated activity of PD-1 inhibitors, there has been speculation regarding the prognostic value of irAEs. Some experts consider irAEs to be a projection of the overall immune response to PD-1 inhibitors, and hence irAEs could be used to gauge the overall tumor response or drug efficacy. However, the value of irAEs as a predictive marker for better patient survival is still debated.

An innovative PD-1 inhibitor Sintilimab is a human immunoglobulin G4 (IgG4) monoclonal antibody that can specifically bind PD-1 molecules on the surface of T Cells, thereby blocking the PD-1/PD-L1 pathway leading to tumor immune tolerance. In December 2018, Sintilimab was approved by Chinese National Medical Products Administration for the treatment of relapsed or refractory classical Hodgkin's lymphoma after at least second-line systemic chemotherapy. In November 2019, Sintilimab became the first PD-1 inhibitor in China to be listed in the National Medical Insurance Directory. In 2021, sintilimab was added as an indication in combination with other drugs for the first-line treatment of unresectable advanced or recurrent squamous non-small cell lung cancer, and unresectable or metastatic hepatocellular carcinoma. More than two dozen clinical studies (more than 10 of which are registered clinical trials) are currently underway worldwide to evaluate the antitumor effects of sintilimab in various solid and hematologic tumors (Guo et al., 2022; Hao et al., 2022; Wang et al., 2022; Zeng et al., 2022).

The case that we reported a Chinese old patient diagnosed with metastatic pancreatic cancer. Two months after Sintilimab treatment, the patient occurred severe immune colitis. The patient was diagnosed with immune ureteritis after 8 months of treatment. The irAEs improved after timely recognition and correct intervention by the clinician and clinical pharmacist. After the first-line treatment of Sintilimab plus gemcitabine combined with pancreatic HIFU, the patient achieved a remarkable benefit of 11-month PFS and 20-month OS (Figure 1).

2 Case presentation

A 80-year-old female was diagnosed with pancreatic cancer (cTxNxM1, Ⅳ, liver metastasis) in November 2019. Contrast-enhanced Magnetic Resonance Imaging (MRI) of the upper abdomen showed pancreatic head and uncinate process lesions and hepatic hemangioma in S2 and S3 segments (Figures 2A,C). Needle biopsy of pancreas revealed heterotypic cells, highly suspicious malignancy. The patient had a history of diabetes and was taking clonazepam as an antidepressant. On November 22, the patient underwent pancreatic HIFU, and was treated with gemcitabine (D1, D15, 1.2g, q4w). On December 16, sintilimab (a PD-1 monoclonal antibody) 200 mg was administered as the first-line treatment. On 15 January 2020, the patient underwent pancreatic HIFU. On January 16, February 5 and 22, gemcitabine (D1, 1g, q4w) plus sintilimab (D1, 200mg, q3w) were administered. Two months after sintilimab treatment, the patient developed severe diarrhea with >8 stools/d, weight loss of 2 kg and obvious abdominal pain. The patient was clinically diagnosed with immune colitis (CTCAE grade 3). The immune colitis recovered from the intravenous administration of methylprednisolone (2 mg/kg/d) for 5 days. The MRI imaging conferred significant tumor shrinkage (Figure 2B) and reduction of liver metastases on March 11(Figure 2D). The therapeutic evaluation of the patient was partial response (PR). On April 3 and 29, sintilimab plus gemcitabine were continued. Tumor markers significantly decreased (Figures 2E,F). Stable disease (SD) was evaluated. After treatment with sintilimab and gemcitabine on May 25, the patient was diagnosed with thrombocytopenia (CTCAE grade 2). Tebiol was given to raise platelets which led to a complete recovery within 5 days. After the treatment of sintilimab (D1, 200 mg) on July 31, the patient had frequent and urgent urination. Urine routine showed leucocyte esterase ++, protein +, red blood cell 153/μL and white blood cell 9243/μL. The infection of urinary tract was considered, but the patient had no fever, and repeated urine cultures of bacteria and fungi were negative. And pelvic CT of the patient was normal on May 21 during the earlier period of sintilimab treatment. On August 5, the patient's depression worsened. After the consultation with the psychologist, mirtazapine was added to improve her depression. On August 14, pelvic CT (Figure 3A) showed that the wall of middle and lower sections of the left ureter was slightly

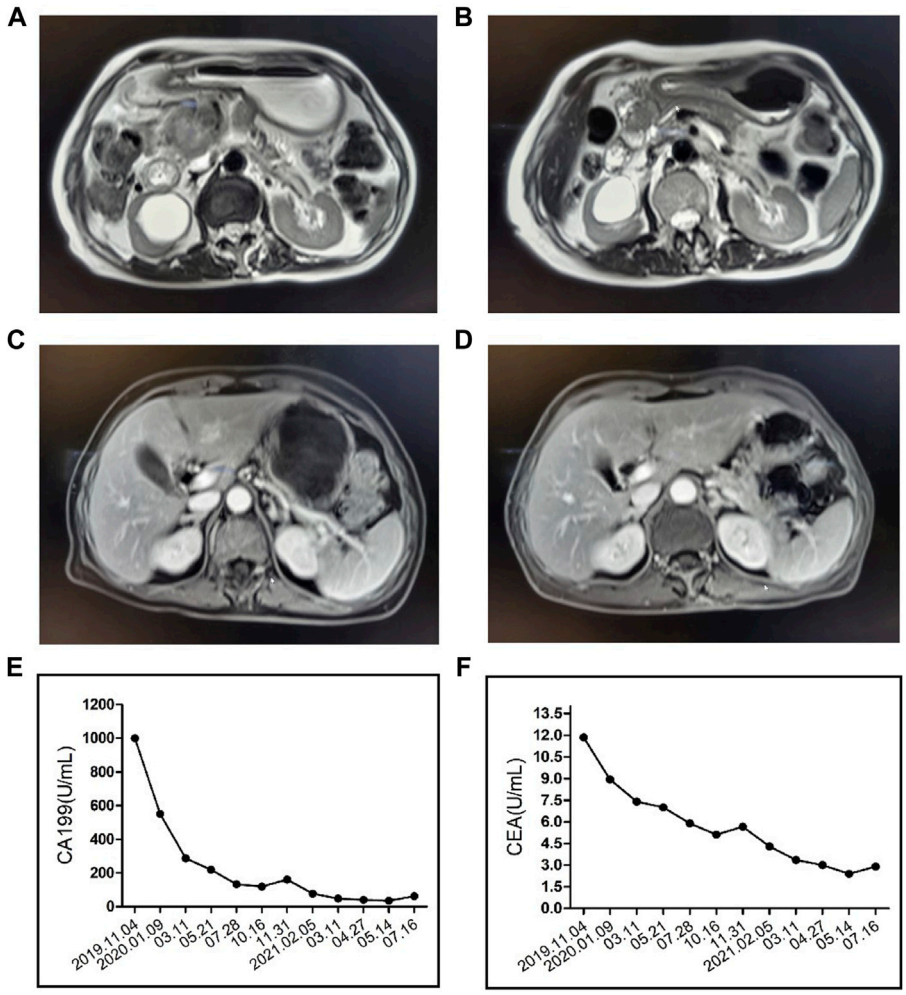
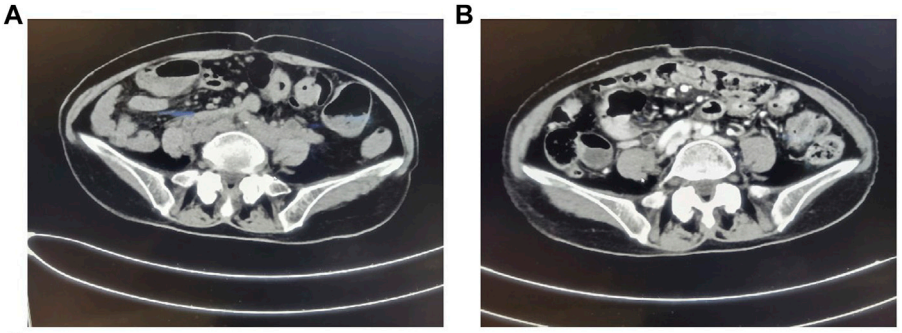


FIGURE 2
Contrast-enhanced Magnetic Resonance Imaging (MRI) of the upper abdomen showed the patient's pancreatic carcinoma before **(A)** and after **(B)** the treatment of PD-1, HIFU and Gemcitabine. MRI showed the patient's liver metastasis before **(C)** and after **(D)** the treatment. CA199 **(E)** and carcinoembryonic antigen (CEA) **(F)** index of the patient.

thickened and dilated with fluid. Anti-infective treatments of ceftriaxone and levofloxacin were given for 2 weeks, and routine urine examinations showed no significant improvement. Thus the patient was diagnosed with PD-1 induced immune ureteritis. Sintilimab was discontinued and dexamethasone (5mg, qd, ivgtt) was given for 6 days. On August 22, routine urine examination markedly improved (Figure 3C). The patient's urinary symptoms significantly relieved and CT imaging of urinary tract (Figure 3B) also showed improvement. The transurethral laser lithotripsy of ureteral/pelvis was performed on September 21. The patient's second-line treatment regime was albumin paclitaxel (D1, 200 mg, Q3W) and HIFU. On November 6, enhanced CT of upper abdomen showed the tumor enlarged and liver metastases progressed. And tumor markers elevated (Figures 2E,F). The PFS of this patient was 11 months. On 5 January 2021, the patient's third-line treatment regime was bevacizumab (D1, 200 mg) and oxaliplatin (D2, 100 mg). On March 11, enhanced CT of the upper abdomen showed that the pancreatic lesions enlarged and tumor progressed. CT of the pelvis showed no obvious abnormality. On March 17, the patient restarted immunotherapy. Bevacizumab (D0, 200 mg) + sintilimab (D1, 200 mg) + gemcitabine (D1, 1.0 g, Q2W) were given, and the patient's vomiting reaction was severe. The patient's treatment regime was adjusted to Gemcitabine (D1, 1 g, Q2W) and HIFU. In June, the patient developed anorexia, eating less, fatigue, and falling. The patient did not cooperate to take antidepressant drugs, and the medication adherence was poor. On July 6, the patient was treated with bevacizumab (D1, 100 mg) and erlotinib (150 mg) which led to a rash with pruritus that was intolerable. The patient died on 4 August 2021, and the main diagnosis of death was cachexia and depression. The OS of this patient has exceeded 20 months.

3 Discussion

We reported a case of a Chinese old female diagnosed with metastatic pancreatic cancer. After first-line treatment of Sintilimab plus gemcitabine combined with pancreatic HIFU, the patient achieved a remarkable benefit of 11-month PFS. Weiss GJ et al. indicated that gemcitabine plus pembrolizumab as the first-line treatment in pancreatic adenocarcinoma could achieve an improved PFS and OS of 8.1 months and 15 months, respectively (Weiss et al., 2018). In



C

Time	Leucocyte esterase	Protein	Red blood cell/μL	White blood cell /μL	The treatment process
2020.08.01	+++	+	153	9243	
08.15	+++	++	1760	14145	After 2 weeks of anti-infective therapy
08.22	+	-	274	154	After 6 days of dexamethasone treatment

FIGURE 3
Pelvic CT imaging showed the patient's immune ureteritis before **(A)** and after **(B)** the treatment of dexamethasone. The urine examination **(C)** of the patient.

contrast, the combination of HIFU in the patient we reported resulted in the prolongation of PFS and OS by 3 and 5 months, respectively. A patient with metastatic pancreatic adenocarcinoma treated with FOLFIRINOX and gemcitabine plus nab-paclitaxel switched to irinotecan liposomal, at the same time was started on maintenance pembrolizumab and olaparib with no progression on CT surveillance for 8 months (Zhao et al., 2022). By contrast, the patient we reported achieved a remarkable benefit of 11-month PFS after first-line treatment of Sintilimab plus gemcitabine combined with pancreatic HIFU. To sum up, HIFU has a promising modality for the extension of survival time in advanced pancreatic patients. The ability of HIFU plus gemcitabine to control tumor outgrowth was moderately enhanced by adjuvant treatment with anti-PD-1 *via* adaptive immunity (Sheybani et al., 2020).

Two months after Sintilimab treatment, the patient occurred severe immune colitis. And the patient was diagnosed with immune ureteritis after 9 months of treatment. Chinese patients with advanced pancreatic cancer receiving immune therapy as a first-line treatment had prolonged survival compared with those receiving it as a second-line or multiple-line treatment, but the difference was not statistically significant. The immune-related adverse events that occurred were hypothyroidism, diarrhea, and rash (Sun et al., 2018). While the patient reported here presented immune colitis and rare immune ureteritis after PD-1 treatment. Chronic immune-mediated diarrhea can develop among patients with a more aggressive disease course and chronic features on colon histology. It likely reflects a prolonged immune checkpoint inhibitor effect and is associated with better cancer outcome and overall survival (Zou et al., 2020). Gastrointestinal-irAEs are associated with improved OS and PFS in patients with metastatic melanoma. Furthermore, higher grades of diarrhea are associated with even better patients' OS rates (Abu-Sbeih et al., 2019). The patient we reported occurred immune colitis and rare immune ureteritis while also achieving a remarkable benefit of PFS and OS. The underlying mechanisms need to be further explored. It is worth noting that the elderly patient also suffered from depression. The combination treatment of Sintilimab, gemcitabine and HIFU for pancreatic cancer prolonged the patient's OS, but the uncontrolled depression worsened the survival to rare and regrettable 20-month OS. Major depression is associated with worse survival in patients with common cancers (Walker et al., 2021). The association and clinical implications require further study.

This case demonstrated that PD-1 and HIFU are feasible and promising treatments for advanced pancreatic cancer. The first-line treatment of Sintilimab plus gemcitabine combined with HIFU demonstrates a potential therapeutic effect on metastatic pancreatic carcinoma with tolerable adverse reactions.

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

QX, HQ, and YZ contributed to the implement of the treatment. HF, YSQ, CCZ, and YYY contributed to the collection and preparation of clinical data and graphic presentation. C-XN, FH, and D-JL drafted

the manuscript. QX and F-MS supervised and reviewed the writing. All authors 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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The correlation between the costs and clinical benefits of PD-1/PD-L1 inhibitors in malignant tumors: An evaluation based on ASCO and ESMO frameworks

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Background: Life expectancy for patients with malignant tumors has been significantly improved since the presence of the programmed cell death protein-1/programmed cell death protein ligand-1 (PD-1/PD-L1) inhibitors in 2014, but they impose heavy financial burdens for patients, the healthcare system and the nations. The objective of this study was to determine the survival benefits, toxicities, and monetary of programmed cell death protein-1/programmed cell death protein ligand-1 inhibitors and quantify their values.

Methods: Randomized controlled trials (RCTs) of PD-1/PD-L1 inhibitors for malignant tumors were identified and clinical benefits were quantified by American Society of Clinical Oncology Value Framework (ASCO-VF) and European Society for Medical Oncology Magnitude of Clinical Benefit Scale (ESMO-MCBS). The drug price in Micromedex REDBOOK was used to estimate monthly incremental drug costs (IDCs) and the correlation between clinical benefits and incremental drug costs of experimental and control groups in each randomized controlled trial, and the agreement between two frameworks were calculated.

Results: Up to December 2022, 52 randomized controlled trials were included in the quantitative synthesis. All the randomized controlled trials were evaluated by American society of clinical oncology value framework, and 26 (50%) met the American society of clinical oncology value framework "clinical meaningful value." 49 of 52 randomized controlled trials were graded by European society for medical oncology magnitude of clinical benefit scale, and 30 (61.2%) randomized controlled trials achieved European Society for Medical Oncology criteria of meaningful value. *p*-values of Spearman correlation analyses between monthly incremental drug costs and American society of clinical oncology value framework/European society for medical oncology magnitude of clinical benefit scale scores were 0.9695 and 0.3013, respectively. In addition, agreement between two framework thresholds was fair (κ = 0.417, p = 0.00354).

Conclusion: This study suggests that there might be no correlation between the cost and clinical benefit of programmed cell death protein-1/programmed cell death protein ligand-1 inhibitors in malignancy, and the same results were observed in subgroups stratified by drug or indication. The results should be a wake-up call for oncologists, pharmaceutical enterprises and policymakers, and meanwhile advocate the refining of American Society of Clinical Oncology and European Society for Medical Oncology frameworks.

KEYWORDS

PD-1/PD-L1 inhibitors, malignant tumors, cost-benefit analysis, ASCO-VF framework, ESMO-MCBS framework

Introduction

Survival benefits of patients with a malignant tumor have been improved significantly over the years, partially attributed to the employment of novel anti-cancer therapies. Recent success in immunotherapy propels cancer treatment to an exciting new era after traditional chemotherapy and targeted therapy (Chen et al., 2019). To date, approximately 4000 clinical trials focusing on programmed cell death protein-1/programmed cell death protein ligand-1 (PD-1/PD-L1) inhibitors have been carried out in at least 20 types of cancer, including both solid and hematological tumors; the total number of subjects worldwide is more than 20,000 (Chen et al., 2020). For the moment, approximately six PD-1/PD-L1 inhibitors are commonly used in clinical practice: Nivolumab, Pembrolizumab, Atezolizumab, Avelumab, Durvalumab, and Cemiplimab. These PD-1/PD-L1 inhibitors are demonstrated to have the preeminent potential for long-term survival, but along with dramatic high drug costs. Although the rapid development of novel therapies has provided insights into the future direction of treatments for malignancy, the high cost of cancer treatment has become a major concern for patients and the society. The financial toxicity may lead to psychosocial distress, poor quality of life (QOL), and worse patient outcomes. Thus, the focus that if the survival benefit and living quality are in proportion to the economics expenditure has been in the spotlight (Goulart, 2016).

However, it is always hard to objectively quantify therapy value and clinical benefit. It is commendable that the American Society of Clinical Oncology Value Framework (ASCO-VF) (Schnipper et al., 2015; Schnipper et al., 2016) and the European Society for Medical Oncology Magnitude of Clinical Benefit Scale (ESMO-MCBS) (Cherny et al., 2017a; Cherny et al., 2017b) have been proposed as evaluation frameworks to analyze survival, toxicity, and QOL of solid tumor patients. These two frameworks were both proposed successively in 2015 and refined in 2016 and 2017, respectively. Since the release of the first research refer to evaluate the clinical benefit and expenditure of solid tumor by using ASCO-VF and ESMO-MCBS frameworks in 2017, several similar studies were conducted in France, Canada, Switzerland Korea and so on (Del Paggio et al., 2017; Vivot et al., 2017; Saluja et al., 2018; Vokinger et al., 2020; Ha et al., 2022). The aforementioned studies aimed to evaluate the value of anti-cancer drugs and help patients and physicians to draw informed comparisons between different cancer treatments. Two tools are increasingly being used to assess the extent to which the magnitude of clinical benefit in these settings is associated with modern drug costs.

Thus, this study attempted to employ the ASCO-VF and the ESMO-MCBS to describe the clinical benefit of all approved PD-1/PD-L1 inhibitors for treating malignant tumors, and calculated the unit time cost of each agent, so as to explore a correlation between clinical benefit and price of drugs, likewise the correlation analyses in the subgroups of different agents or indications. Furthermore, consistency evaluation of two value frameworks was also computed.

Materials and methods

Identification of study cohort

PubMed was searched from the inception of a database to December 2022 to identify all the phase III randomized controlled trials (RCTs) in treatment with malignant tumors involving approved PD-1/PD-L1 inhibitors (Nivolumab, Pembrolizumab, Atezolizumab, Durvalumab, Avelumab and Cemiplimab), by the terms of drug names and clinical trials [i.e., nivolumab (Title/Abstract) AND clinical trial (Title/Abstract)]. Phase III RCTs registered on the clinicaltrials.gov website were also incorporated. Abstracts and methods of each trial were reviewed to identify the eligible cohort of trials according to inclusive criteria that RCTs could be analyzed with ASCO-VF or ESMO-MCBS, and the clinical benefit of the experimental groups should be preferred over the control groups. Analyses of patient-reported outcomes only assessing the QOL of the corresponding RCTs were also included. The following research were not taken into account: the secondary, subset, or systematic reviews; phase I, II or IV trials or animal studies; trials focused on other objectives including pharmacokinetics, drug dosing schedules, iconography, biomarkers, modeling, etc; non-trial-based papers like trial introduction; and trials that written by non-English articles. The study was conducted independently by two authors (YH and SL), and discrepancies were resolved by consensus in the presence of a third investigator (XW).

ASCO-VF and ESMO-MCBS scoring

Gains in a survival endpoint and adjustments by toxicity and QOL in scores or grades were quantified by ASCO-VF (Version 2), or ESMO-MCBS (Version 1.1), or both if data allowed. The clinical benefit score of ASCO-VF is based on the point estimate of the hazard ratio (HR) of a couple of clinical endpoints covering overall

survival (OS), progression-free survival (PFS), and response rates (RR), which is subtracted from 1 and the result is multiplied by 100 to derive the preliminary score. For toxicity assessment, both the number of the occurred case and the frequency (i.e., ≥10%, <10%, ≥5%, <5%) of all grades' adverse events are correspondingly assigned "points", which are applied to formulaically figure up the increment of the experimental group against the control group to derive an adjustment of the score (i.e., ±20 points maximum adjustment). For QOL, ASCO-VF allows an award of 10 points if a statistically significant improvement in QOL is reported but no deduction due to detrimental QOL. Besides, ASCO-VF includes bonus points for a "tail of the curve effect" (16–20 points), palliation of symptoms (10 points), and treatment-free intervals (a percentage-calculated improvement). The final ASCO-VF scores are the sum of above items (possible range −20–180) (Schnipper et al., 2016). ASCO-VF does not explicitly define "meaningful clinical benefit" scores, so the median score was used to determine meaningful clinical benefit according to the suggestion of reference (Del Paggio et al., 2017).

In the ESMO-MCBS grading system, the lower limits of the 95% CI of the HR of survival outcomes are used to determine a particular grade in a pre-specified manner, which is downgraded if pre-specified toxic effects are explicitly outlined in the experimental group with specifically, statistically significant incremental rates like "Toxic death >2%," "Cardiovascular ischemia >2%," "Grade 3 neurotoxicity >10%" and so on. For QOL assessment, upgrading or downgrading are allowed base on the improvement or deterioration of QOL. Ultimately, ESMO-MCBS grades are ranked from 1 to 5 for the advanced disease setting, and C, B, or A for the curative setting. ESMO-MCBS defines "meaningful clinical benefit" as a grade of 4, 5 or B, A (Cherny et al., 2017b).

Incremental drug cost

To assess the monthly cost of therapeutic regimen including the cost of all anticancer drugs in the study regimen, we used the United States average wholesale prices (AWP) for drugs from the RedBook (IBM Micromedex, Armonk, NY, United States). Monthly costs were calculated over an average of 28 days based on the dosage schedule in all eligible trials for a patient weighing 70 kg with a body surface area of 1.86 m^2 and creatinine clearance of 100 mL/min (Wan et al., 2019). Ultimately, incremental monthly drug costs between the experimental and control groups were reported. All therapeutic regimens were adjusted to provide the price per 4-week period.

Statistical analysis

Study data like ASCO-VF and ESMO-MCBS scores, and incremental cost were mainly statistically described with median values, the 25th and the 75th percentile basing on treatment purposes, agents or indications. The scores of each trial were presented as a histogram. Spearman's rank correlation coefficient (r) was calculated to assess the association between non-normally distributed data or ordinal data, such as costs and scores, which were showed by scatterplots or boxplots. Mann-Whitney U test was performed to describe the correlation between cost data and clinical benefit thresholds, shown as boxplots. Agreement between ASCO-VF and ESMO-MCBS in clinical benefits of RCTs was calculated *via* Cohen κ statistics, by which the result was between 0 and 1 (0 indicates agreement equivalent to chance and 1 indicates perfect agreement) (Cohen, 1960). No quantized analysis was made for ESMO-MCBS grades in the curative setting RCTs because its grades are non-numerical.

All statistical analyses were conducted in R (version 4.1.0) using ggplot2 (version 3.2.0) for plots. p-values of less than 0.05 were considered statistically significant.

Results

Overview and characteristics of RCTs

A total of 101 RCTs were initially identified. After excluding trials that failing to meet inclusion criteria, 65 papers of 52 phase III RCTs were analyzed containing six PD-1/PD-L1 inhibitors: 17 RCTs for pembrolizumab, 14 RCTs for nivolumab, 14 RCTs for atezolizumab, 4 RCTs for durvalumab, 2 RCTs for avelumab, 1 RCTs for cemiplimab (Figure 1). The RCTs covered 12 indications, among which, 19 RCTs were used for non-small cell lung cancer (NSCLC), 7 for melanoma, 5 for breast cancer, 5 for renal cell cancer, 4 for urothelial cancer, 3 for gastric cancer, 2 for hepatocellular cancer, 2 for head-and-neck squamous cell carcinoma, 2 for small cell lung cancer (SCLC), 1 for colorectal cancer, 1 for glioblastoma, and 1 for malignant pleural mesothelioma. All of eligible papers were listed in the Supplementary Material.

Frameworks scores

Of 52 RCTs, 4 were the curative setting, and the others were the advanced setting. All-inclusive RCTs were eligible for assessment by the ASCO-VF, and among which 49 RCTs were also eligible for ESMO-MCBS assessment.

ASCO-VF scores ranged from 0.40 to 86.71 (Supplementary Table S1). The scores were not normally distributed and therefore, were described in terms of medians and quartiles. The median ASCO-VF score of 52 RCTs was 39.81 (IQR 18.23–56.54), with 26 trials below and 26 trials above (Supplementary Figure S1). For the 48 palliative trials, 24 fell below the threshold and 24 were above the threshold (Median 40.16, IQR 21.16-56.79), whereas for the 4 curative trials, two felled below and the other two were above (Median 24.10, IQR 16.99-36.10).

For the assessment of ESMO-MCBS, among 49 RCTs, 19 trials felled below the "meaningful benefit" score, 30 were above (Supplementary Figure S1). For the 46 palliative trials, 18 fell below and 28 were above the threshold. In the 3 curative trials, 1 fell below and 2 were above the threshold. Median scores and quartiles of RCTs with different indications and agents were presented in Supplementary Table S1.

Relation between cost and value of drug

The incremental monthly drug costs (the cost of the experimental group minus the cost of the control group) of PD-

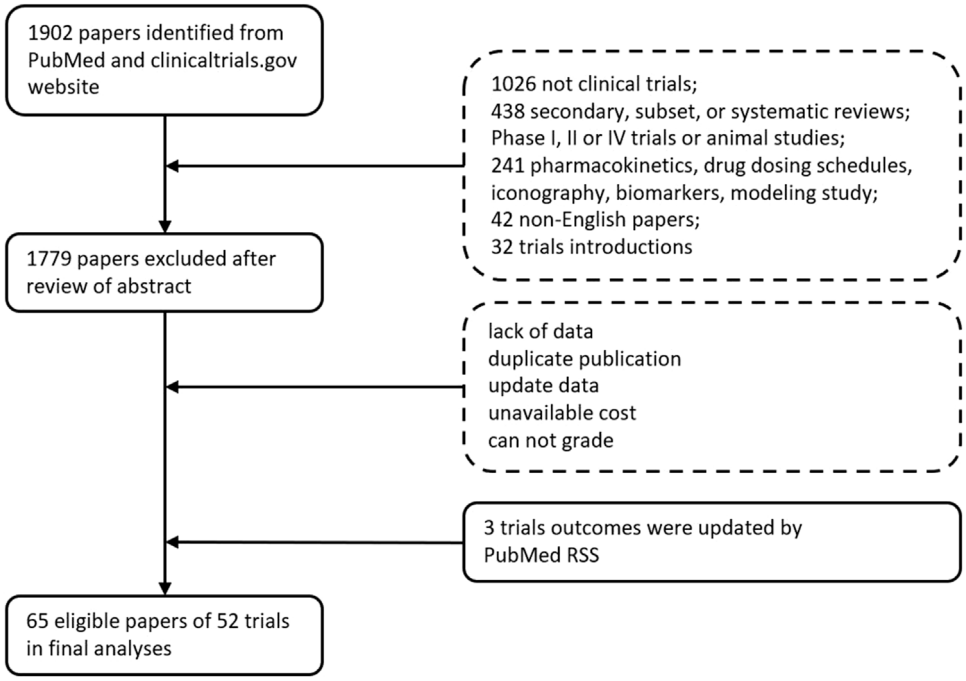

FIGURE 1
Identification of RCTs of all therapy in six immune checkpoint inhibitors.

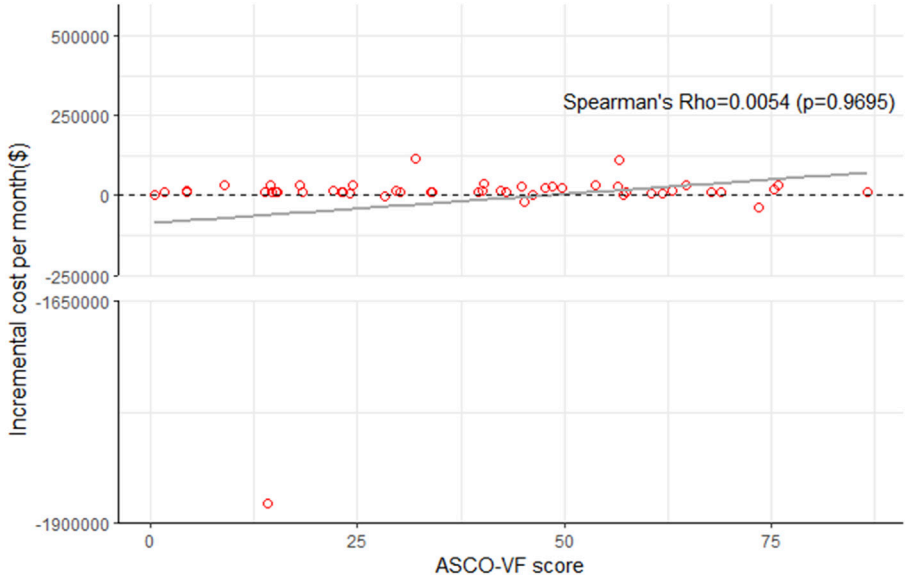

FIGURE 2
Scatterplot of six immune checkpoint inhibitors between ASCO-VF scores and incremental cost per month in all RCTs.

1/PD-L1 inhibitors and the ASCO-VF score were not statistically significant correlated in all trials (Spearman's $\rho = 0.0054$, $p = 0.9695$, Figure 2), the subgroup of palliative treatments ($\rho = -0.0396$, $p = 0.3946$), and curative treatments ($\rho = 0.6324$, $p = 0.184$) (Supplementary Figure S2).

For ESMO-MCBS grades, no statistically significant association was also noted in the palliative setting ($\rho = -0.0788$, $p = 0.3013$) (Figure 3). Stratified by indications or drugs, no statistically correlations were found between either framework and costs ($p > 0.05$) (Figure 4). Correlation analysis could not be conducted in

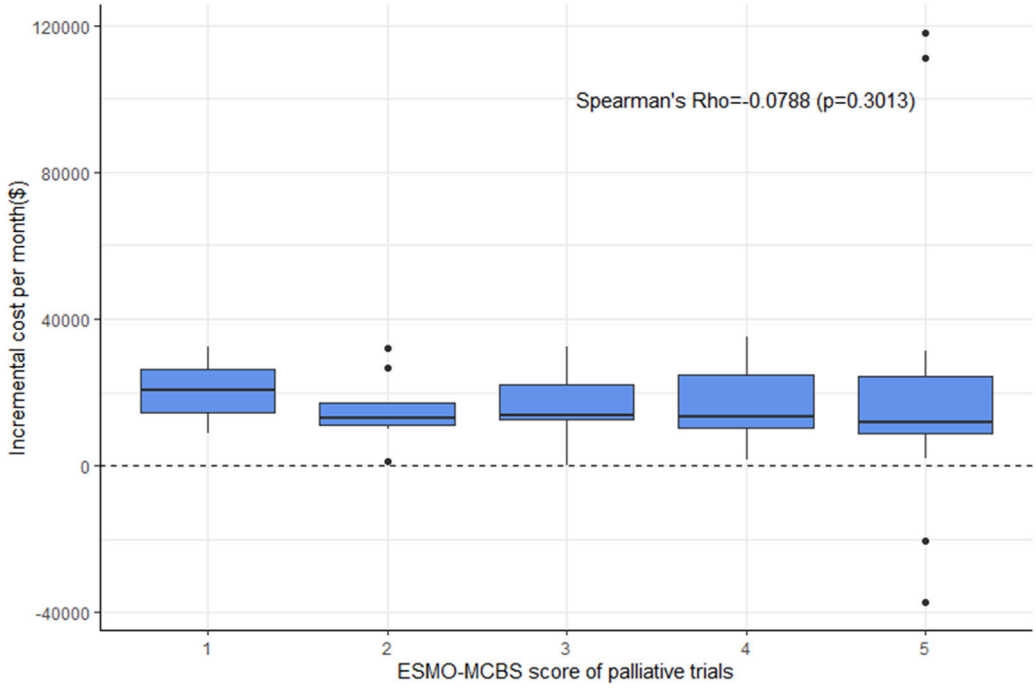

FIGURE 3
Boxplot of correlation between ESMO-MCBS scores and incremental cost per month in palliative trials.

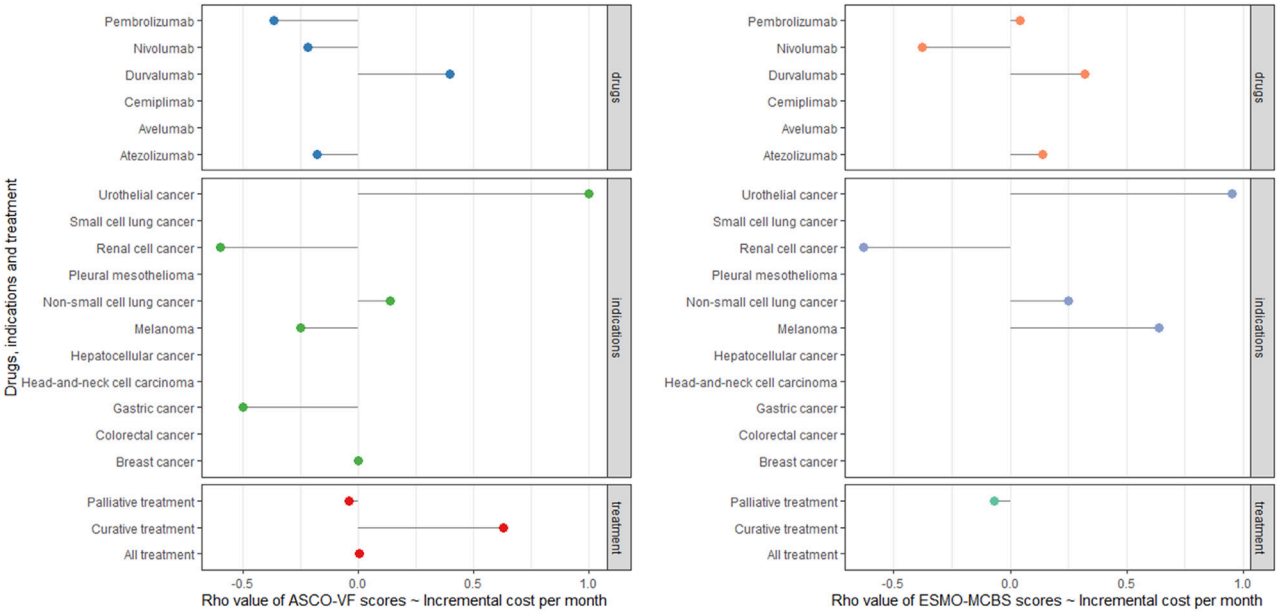

FIGURE 4
Cleverland of correlation analyses among ASCO-VF scores, ESMO-MCBS scores and incremental cost in six PD-1/PD-L1 inhibitors and 11 indications.

curative setting due to its non-consecutive numerical data of grades C, B and A.

The incremental monthly drug costs of trials that did not meet the ASCO-VF threshold for meaningful benefit was slightly lower than that of met the meaningful benefit [$12504 (IQR 11902 to 15451) vs. $13392 (IQR 9391 to 26681); p = 0.8444], while the opposite result was observed when used ESMO-MCBS framework [not met the threshold $12948 (IQR 10435 to 23517)vs. met the

threshold $12499 (IQR 9629 to 24875); $p = 0.9014$] (Supplementary Figure S3). Neither result was statistical significance.

Agreement of frameworks

When comparing the RCTs scores using the framework-specified thresholds, Cohen κ statistic was calculated as 0.417 ($p = 0.00354$), which suggested a moderate agreement between ASCO-VF and ESMO-MCBS thresholds. In subgroup analyses, within the palliative subset, the κ score (0.421, $p = 0.00426$) was similar to that in the total cohort, whereas in curative setting of trials, the κ score was weaker than the total cohort (0.333, $p = 0.564$).

Discussion

Cancer drug innovation has been accelerating since entering the 21st century. The number of novel cancer drugs approved in 2005–2015 was over 8 times more than that approved in 1975–1985 (66 vs. 8), and the average annual growth rate of total cancer drug expenditure was 7.6%, 3.6 times more than the average annual growth rate of nominal United States Gross Domestic Product (Lichtenberg, 2020). In the context of limited medical resources, it is essential to evaluate the correlation between clinical benefit and medical expenditure. To the best of our knowledge, this was the first study that applied ASCO-VF and ESMO-MCBS to assess the clinical benefit of all approved PD-1/PD-L1 inhibitors comprehensively. Both frameworks demonstrated that only nearly half of the eligible trials (26 of 52 trials in ASCO-VF and 30 of 49 in ESMO-MCBS) had met the "meaningful clinical benefit" thresholds correspondingly, which suggested that quite a lot of RCTs only demonstrated subtle clinical benefits. Furthermore, there was no statistically significant correlation between drug price and the clinical benefit in all trials, even in the subgroups of different indications/different agents, which revealed that high prices might not definitely yield the equivalent benefit.

Previously, two prior studies showed no significant association between clinical benefit and the price of new FDA-approved anti-cancer drugs with initial indications in the United States from 2000 to 2017, using both ASCO-VF and ESMO-MCBS (Vivot et al., 2017; Vokinger et al., 2020). The result of our study, which focused on PD-1/PD-L1 inhibitors, were consistent with two prior studies and partial presented the weak association between clinical benefit and the drug price in all anti-cancer drugs. One prominent reason attributed to this situation might be that these novel agents are always highly priced by pharmaceutical enterprises within patent protection. As per the Tufts Center for the Study of Drug Development in 1975, pharmaceutical industries expended 100 million dollars for the research and development of the FDA-approved drug, which had surged to $1.3 billion in 2005 stupendously (Kunnumakkara et al., 2019). In order to repay their high and risky investment cost, pharmaceutical companies would charge more for their products, which may be the partial cause for the high sale prices of drugs outweigh their clinical efficacy. Besides, the inaccurate evaluation of drug efficiency is another contributor. Many drugs get approval from the FDA in an expedited regulatory pathway (called accelerated approval) on the basis of existing trial endpoints at that time, which probably exaggerates the clinical benefit and safety of these drugs provisionally. Some drugs or indications were withdrawn from the market after reevaluation in post-marketing studies (Wilson et al., 2013), such as the indications that pembrolizumab in second-line treatment of SCLC, nivolumab in second-line treatment of SCLC, nivolumab in second-line treatment of BRAF-positive melanoma, atezolizumab in urothelial cancer and so forth. A report published by FDA indicated that from 11 December 1992 to 31 May 2017, 5% of 93 indications of oncology were withdrawn in light of post-approval trials results (Beaver et al., 2018), which suggests the accuracy of evaluating anti-cancer deserves more attention. In a word, the high drug cost and the uncertainty of clinical benefit work together to the no association between them.

Two value frameworks were applied in this study, and a moderate agreement was found between them. There is a controversy exist in the agreement of these two frameworks (Cheng et al., 2017; Del Paggio et al., 2017; Vivot et al., 2017; Cherny et al., 2019; Jiang et al., 2020), which are not surprising given the differences in their construction and scoring criteria. First, major factors contributing to discrepancy are different methods of evaluating relative and absolute gain for OS and PFS, applying toxicity penalties, and crediting the tail of the curve gains. By these methods, the ASCO-VF tends to generate lower clinical benefit scores in comparison to ESMO-MCBS. Second, the frameworks differ in their criteria for awarding bonus credits for long-term survival gain. The ASCO-VF criteria awards bonus points on the basis of a 50% or greater improvement at the time point that is twice the comparator median survival time on the survival curves. ESMO-MCBS credits an adjustment grade if there is a long-term plateau in specified time points of the survival curves. Third, both frameworks award bonus scores for treatments that reduce toxicity, but their approaches differ, which have been described in methods. Distinctness of awarding bonus in ASCO-VF and ESMO-MCBS generated the gap in clinical benefit scores as well. Although the tools are imperfect, they have been at the forefront of evaluating the relation between clinical benefit and cost for many years.

From the perspective of society, growing expenditures on anticancer drugs can potentially occupy the investment of other life–saving medicine, and contribute to the unbalanced allocation of medical resources. Virtually, many drugs like anti-cardiovascular diseases drugs are available as generics or "me-too" that are defined a new pharmaceutical compound with a known pharmaceutical class of treatment, and increasing competition consequently led to diminishing overall costs in these pharmaceutical companies, while most the anticancer agents are the first-in-class agents. During 1970–2000, the life expectancy of Americans increased on average by 6 years; only 6 months were attributed to antineoplastic therapies, while over 4 years were attributed to cardiovascular disease (Lenfant, 2003). A horrendous disequilibrium between prices and survival benefits causes a dire socioeconomics cost and puts a substantial burden on the medication budgets of public health organizations. Therefore, it has profound meaning to assess the survival benefits and economy investment to re-allocate medical resources.

This study has several limitations. Firstly, we only evaluated available trials published to assess the ASCO-VF and the ESMO-

MCBS scores to date. Within a trial, outcomes of long-term follow-up and the further pooled estimate of efficacy result would evolve with time, which lead to the dynamics of clinical benefit scores of drugs (Schnipper et al., 2016). Similarly, due to the data availability, agents that have not been approved or whose wholesale prices are not accessible were not included in our study. We also excluded studies written in non-English languages. All these incomplete and inconclusive data would give rise to biases in subsequent analysis. Secondly, different from ESMO-MCBS, ASCO-VF does not provide its own "meaningful clinical benefit" threshold, so we use the median value of ASCO-VF scores for comparison according to the reference, which may partly contribute to the moderate agreement between ASCO-VF and ESMO-MCBS. In addition, in this study, only monthly incremental drug costs were considered, but treatment duration might affect the total cost differences between the experimental group and the control group, whichever probably have predefined courses. However, most of the included trials were palliative treatment, and the calculated incremental costs likely represent approximately 90% of the total treatment course increment cost, so monthly incremental drug costs were a close approximation reflection so long as response to treatment continues (Mittmann et al., 2009; Bradbury et al., 2010; Del Paggio et al., 2017). Thirdly, due to the limitation of sample size and research design, many phase III clinical trials in malignancy have relatively wide 95% CI. Based on the instructions of these two frameworks, point estimation of HRs was utilized in ASCO-VF framework tool, which would add uncertainty to the scores. The ASCO-VF should be planned revised and dynamically updated upon recognition of expanding needs and shortcomings identified. While in the latest version of ESMO-MCBS framework (Version 1.1), the lower limit of 95% CI is adopted for a required HR, and the absolute survival gain is taken into account, potentially balancing this uncertainty. Finally, understanding degree of frameworks among different investigators would be reflected in the research. Although this analysis was performed by three investigators, some trivial discrepancy could not be averted. A modified framework or updated trial results are expected to assist in evaluating the cost-benefit of drugs accurately, and shared decision making regarding the options available to oncologists and patients.

Conclusion

This research indicated that on account of ASCO-VF and ESMO-MCBS frameworks, no correlation between the costs and clinical benefits of PD-1/PD-L1 inhibitors was present in treating malignant tumors, and the same results were observed in subgroups stratified by drugs or indications. In addition, the agreement between two framework thresholds was moderate. The result suggests that a comprehensive cost-benefit assessment of novel cancer drugs should guide oncological drug approval in public healthcare organizations, and methods to control and limit drug cost should be coordinated among healthcare providers, pharmaceutical companies, and policymakers. Meanwhile, the refining of ASCO and ESMO frameworks might be addressed to facilitate the standard assessment of clinical benefit of anti-cancer drugs.

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

SL performed conceptualization, formal analysis, software, original draft preparation, review and editing. YH performed data curation, formal analysis, original draft preparation. LD performed formal analysis. ML, performed conceptualization. YW performed formal analysis. DG performed data curation and resources. WW and DN performed data curation. SL performed software. XH performed data curation. XX performed funding acquisition. XW performed conceptualization, supervision, review and editing. All authors read and approved the final 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1114304/full#supplementary-material

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Comparison of the efficacy and safety of third-line treatments for advanced gastric cancer: A systematic review and network meta-analysis

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Background: Many options for third-line treatment of advanced gastric cancer (GC) or gastroesophageal junction carcinoma (GEJC) have been developed. Therapies including immunotherapy (nivolumab), chemotherapy (irinotecan, FTD/TPI), targeted therapy (apatinib), and antibody drug conjugates (ADC) have shown to increase the survival rates in patients, but few studies have compared the relative efficacy of these treatments. Here, we compared the efficacies of these regimens using network meta-analysis (NMA) to provide guides in selecting the best regimen and formulating a precise individualized treatment plan.

Methods: The published RCTs of phase II/III in PubMed, the Cochrane Central Register of Controlled Trials, and Embase were searched. The median overall survival (mOS) was the primary outcome of NMA, and the other outcomes were median progression-free survival (mPFS), disease control rate (DCR) (proportion of patients with confirmed CR, PR, or stable disease (SD)) and incidence of grade 3 or above adverse events (≥3AEs).

Results: Five phase II/III RCTs involving 1674 patients and 7 treatment regimens were analyzed. It showed that Trastuzumab Deruxtecan (DS-8201) prolonged the OS of patients significantly comparing with chemotherapy (HR: 0.59; 95% CI: 0.39-0.89) for the overall population. DS-8201 (HR: 0.27; 95% CI: 0.17-0.42) and chemotherapy (HR: 0.57; 95% CI: 0.47-0.7) improved the PFS significantly over nivolumab. Apatinib (RR: 3.04; 95% CI: 1.65-5.95) and DS-8201 (RR: 2.67; 95% CI: 1.51-4.83) were more effective than nivolumab in improving DCR. DS-8201 achieved greater OS benefits compared to chemotherapy (HR: 0.59; 95% CI: 0.39-0.88) for patients who were HER2-positive. We ranked the Bayesian surface under the cumulative ranking curve according to OS benefit, and showed that ADC ranked first for the general patient population and for patients with a HER2-positive diagnosis, intestinal histopathology, previous gastrectomy history, gastric origination cancer, ages over 65 and ECOG PS=0/1, followed by nivolumab and apatinib. For patients with GEJC, nivolumab ranked first.

Conclusions: Nivolumab, apatinib, chemotherapy, and ADC all improved the OS of GC/GEJC patients significantly. ADC may be the best option for the overall population of GC, as well as for patients with HER2-overexpression, intestinal

histopathology, previous gastrectomy history, gastric origination cancer, ages over 65 and ECOG PS=0/1, followed by nivolumab and apatinib. Nivolumab may be the first treatment option for GEJC patients.

Systematic review registration: https://www.crd.york.ac.uk/prospero, identifier CRD42022364714.

KEYWORDS

gastric cancer (GC), gastroesophageal junction carcinoma (GEJC), third-line treatment, nivolumab, apatinib, ADC, chemotherapy, network meta-analysis (NMA)

Introduction

GC and GEJC have become one of the most frequently diagnosed malignant tumors in recent years, and they ranked the fourth in tumor-caused death (1), with an incidence of 29.3/100,000 and a mortality rate of 21.2/100,000 in China (2). Despite the significant progress in the options for effective surgical and systemic treatments, the overall 5-year survival rate of GC remains at below 30%, and the median OS of advanced gastric cancer (AGC) is only 9-10 months (3, 4). The development of more effective multidisciplinary evaluation and treatments for GC/GEJC is needed.

At present, first-line standard treatments recommended by National Comprehensive Cancer Network (NCCN) guidelines are fluorouracil-based options, combined with standard chemotherapy using platinum and/or taxane, and with or without anti-HER2 drugs depending on HER2 expression status (5). In addition, depending on the expression status of PD-L1, immunotherapy options may be added as well. Second-line treatment is mostly recommended as monotherapy. However, third-line treatment includes many different options after second-line treatment fails. At present, options for third-line treatment of GC or GEJC include targeted therapy (apatinib), immunotherapy (nivolumab), and chemotherapy (irinotecan, FTD/TPI) (6, 7). Despite the survival benefits of all these regimens for GC patients, the objective response rate (ORR) of tumors remains low (2.84%-11.6%) (8–11). Surprisingly, some new ADC drugs have shown great efficacy and safety. For example, trastuzumab deruxtecan (DS-8201) has emerged in recent years as an effective treatment for HER2-positive GC patients. Although DS-8201 may cause interstitial lung disease in some patients with an incidence rate of approximately 10%, its safety profile remains manageable (12). The new ADC drug RC48 is produced by coupling recombinant human anti-HER2 antibody with monomethyl auristatin E (a microtubule inhibitor) through a cleavable linker. In some RCTs, RC48 also showed great anti-solid tumor activity against GC. In addition, it has shown high efficacy in patients with low expression of HER2 (IHC 2+/FISH-) and HER2 overexpression (IHC 2+/FISH + or HER2 IHC 3+) (13, 14). 2.5 mg/kg RC48 was administered every two weeks with a single intravenous infusion to treat patients with HER2 overexpression in a phase II single-arm RCT. The treated participants showed a median OS of 7.9 months (95% CI: 6.7-9.9) and a median PFS of 4.1 months (95% CI: 3.7-4.9) (15).

The overall prognosis of advanced GC is relatively poor. Clinical research on traditional chemotherapeutic agents has not identified effective drugs, the choice of targeted drugs is limited, and the efficacy of immunotherapy alone is insufficient. Therefore, we analyzed several third-line treatment options by comparing their efficacy and safety, to provide a guide in choosing the best third-line treatment for GC.

Materials and methods

Literature search strategies

This NMA was performed according to the PRISMA extension statement (Supplementary Table 1). Publication on the third-line treatments for advanced GC/GEJC in PubMed, Embase, and Cochrane Library and Medline ISI (January 1, 2005 to November 31, 2021) were searched using the search strategy shown in the Supplemental Table 2. We also reviewed abstracts of major conferences (2018-2022) including the European Society of Medical Oncology (EMSO), American Society of Clinical Oncology (ASCO), Chinese Society of Clinical Oncology Collaborative Committee (CSCO), and American Association for Cancer Research (AACR).

Inclusion criteria

We selected published English-language reports of RCTs of phase II/III that compared at least two third-line treatment regimens. The patients who were included in the study were required to have advanced (stage IV) GC/GEJC diagnosed histologically. In addition, the hazard ratio (HR) and 95% confidence interval (CI) with OS and PFS were available.

Exclusion criteria

We excluded phase I clinical trials and those with incomplete data reports. Studies that tested only adjuvant therapy, maintenance therapy, or first-line and second-line therapy were also excluded. We also excluded articles related to tumor vaccine treatment.

Data extraction and risk of bias assessment

We first extracted relevant information of included studies, such as study title, publication year, first author, number of study subjects and baseline characteristics, and indicators of OS, PFS, ORR, DCR as well as ≥3AEs. The risk of bias in RCTs was subsequently determined using the Cochrane Risk of Bias Tool, which included the randomization process, missing outcome data, measurement of outcomes, deviation from the intended intervention, and selection of reported outcomes. RCTs were rated as low, high, or some concern of bias based on this evaluation criteria. For non-RCT, we used the Newcastle-Ottawa scale for quality assessment, which include the exposed cohort, non-exposed cohort, ascertainment of exposure, outcome of interest, comparability, assessment of outcome, length of follow-up, adequacy of follow up. A total score of 5 or more is considered high quality study (16). Extraction of the data and assessment of the risk of bias were carried out by two independent investigators (XYX and ZC).

Statistical analysis

Q-test and I^2 statistics were used to assess the heterogeneity among studies. Heterogeneity among studies could be considered statistically significant if $I^2 \geq 50\%$ or $P < 0.05$. If I^2 values were less than 50%, studies could be considered having low to moderate heterogeneity and a random effect model could be applied for statistical analysis (17). For the HR, relative risk (RR), and corresponding 95% CI of the outcome indicators including OS, PFS, DCR, and ≥3AEs, we applied fixed and random models separately to pool and then compare them by the deviance information criterion (DIC). We then chose the fixed model when the difference in DIC between the random and fixed models was less than 5 (18). Bayesian NMA was carried out using the JAGS and GEMTC packages in R.4.2.0 and Markov chain Monte Carlo simulation technology (19). Each analysis involved 150,000 sample iterations with 100,000 burn-in cycles and a thinning interval of 10. In addition, we used tracking maps and Brooks-Gelman-Rubin diagnostics for visual inspection to help determine the model convergence (20). The network diagrams produced with Stata 16.0 showed the comparative relationships between the various treatments more directly. We calculated the surface under the cumulative ranking (SUCRA) curve to estimate the probability that each treatment method was at each rank. A higher SUCRA value represented a greater possibility that a treatment would be treated as the top choice (21).

Results

Network meta-analysis study characteristics

After the screening process as shown in Figure 1, five phase II/III eligible RCTs (10–12, 22, 23) in our review with a total of 1674 patients and 7 different treatments were included. The treatments included immunotherapy (nivolumab), chemotherapy (trifluridine/tipiracil, irinotecan, paclitaxel), targeted therapy (apatinib), and ADC (trastuzumab deruxtecan, (DS-8201) and virtuximab (RC48-ADC)). Table 1 shows the basic characteristics of included RCTs. Our NMA satisfied the assumption of transitivity that the population baseline was stable across studies with different interventions. (Supplementary Figure 9)

Integrated analysis of median overall survival

We integrated and analyzed the mOS of the same treatment regimen from different RCT studies to obtain the pooled OS (pOS) of the currently available third-line treatment. The pOS of apatinib and ADC as third-line treatments for GC/GEJC were 5.59 months (95% CI 3.96–7.21) and 10.12 months (95% CI 5.61–14.62), respectively (Supplementary Figure 4).

Overall outcomes

The relative efficacy between these treatments was compared first, and the network diagram of direct and indirect comparisons of all treatment regimens are presented in Figure 2. In terms of OS (Figure 3A), nivolumab (HR: 0.66, 95% CI: 0.54-0.8) apatinib (HR: 0.61, 95% CI: 0.48-0.78), DS-8201 (HR: 0.41, 95% CI: 0.26-0.64), and chemotherapy (HR: 0.69, 95% CI: 0.56-0.85) were all significantly increased compared with that of placebo. DS-8201 prolonged the OS of patients significantly (HR: 0.59, 95% CI: 0.39-0.89) over chemotherapy. The SUCRA value of DS-8201 (0.98) was the largest in OS, indicating that it most likely ranked first, followed by apatinib (0.63) and nivolumab (0.49) (Supplementary Figure 1A). The PFS of placebo was significantly shorter than that of nivolumab (HR: 1.67, 95% CI:1.35-2.06), apatinib (HR: 2.66, 95% CI: 2.04-3.46), DS-8201 (HR: 3.47, 95% CI: 2.35-5.91), and chemotherapy (HR: 1.75, 95% CI: 1.44-2.14). Furthermore, the PFS of chemotherapy was shorter than that of apatinib (HR: 1.52, 95% CI: 1.09-2.11) and DS-8201 (HR: 2.13, 95% CI: 1.4-3.21) (Figure 3A). The SUCRA value of DS-8201 (0.97) was higher than that of apatinib (0.77) and chemotherapy (0.41) in PFS. For DCR (Figure 3B), nivolumab (RR: 1.61, 95% CI: 1.18-2.28), apatinib (RR: 4.9, 95% CI: 2.96-8.89), DS-8201 (RR: 4.3, 95% CI: 2.75-7.14), and chemotherapy (RR: 3.09, 95% CI: 2.09-4.85) were shown to have significantly better efficacy over placebo. In addition, apatinib (RR: 3.04, 95% CI: 1.65-5.95), DS-8201 (RR: 2.67, 95% CI: 1.51-4.83) and chemotherapy (RR: 1.92, 95% CI: 1.14-3.31) were

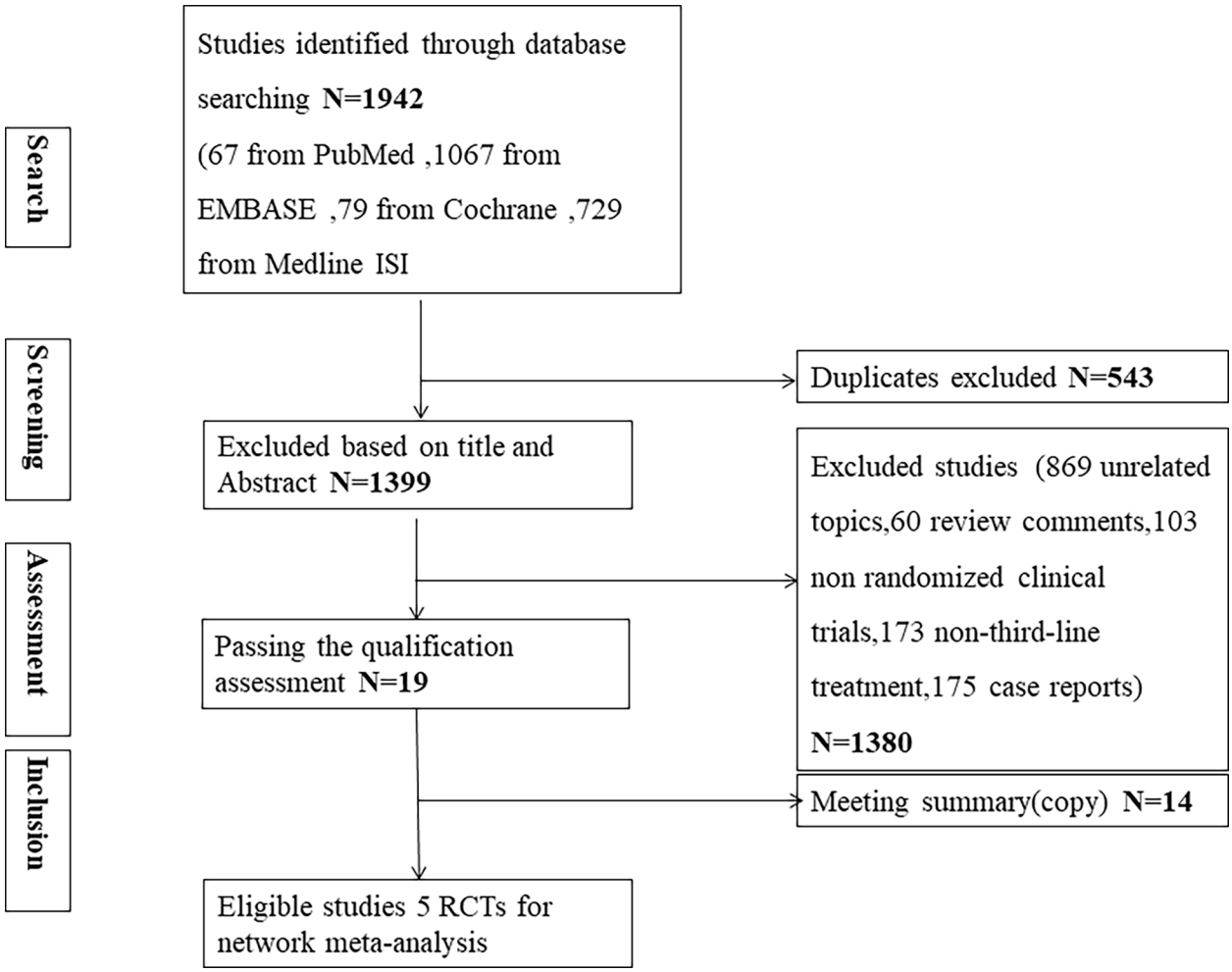

FIGURE 1
Study selection.

superior to nivolumab. The SUCRA value for apatinib (0.89) was the largest in DCR, followed by DS-8201 (0.84). In terms of ≥3 AEs (Figure 3B), nivolumab (RR: 2.82, 95% CI: 1.36-6.86) and chemotherapy (RR: 4.07, 95% CI: 2.78-6.31) were associated with higher incidence rates of adverse events than placebo. According to the statistics of the incidence of ≥3 AEs for various treatments, hypertension(5.4%) and hand-foot syndrome(7.6%) were the most common adverse events for apatinib, while the incidence of hematological toxicity and gastrointestinal-related adverse events was low. As for DS-8201, the incidence of leukopenia (21%) and anemia (38%) were relatively high. (Supplementary Figures 3)

NMA of different HER-2 expression status subgroup

According to the HER2 expression status of advanced GC patients, NCCN guidelines define HER2-overexpression as IHC2+ and IHC3+, in which IHC3+ and IHC2+/FISH+ are HER2-positive and IHC1+ is HER2-negative (6). Nivolumab (HR: 0.38, 95% CI: 0.22-0.66) and DS-8201 (HR: 0.45, 95% CI: 0.23-0.89) achieved significant OS benefits in patients who were HER2-positive compared to placebo. Furthermore, DS-8201 (HR: 0.59, 95% CI: 0.39-0.88) significantly prolonged the OS of patients compared with chemotherapy (Figure 3C). The SUCRA values of DS-8201 (0.85) and nivolumab (0.8) were significantly higher than that of chemotherapy (0.29) in HER2-positive patients (Supplementary Figure 1). For patients with HER2 IHC2+/FISH- or IHC1+, the OS of placebo was shorter than that of nivolumab (HR: 1.41, 95% CI: 1.14-1.75) and chemotherapy (HR: 1.61, 95% CI: 1.23-2.1). However, the SUCRA value of nivolumab (0.61) was still higher than that of chemotherapy (0.39). Because Destiny-Gastronomy 01 (2020) (12) did not document the HR values of patients who were HER2-positive (IHC2+/FISH-) and HER2-negative (IHC1+), we cannot further compare the relative efficacy of DS-8201 with other treatments. However, the results of the exploratory cohort study of DS-8201 by DESTINY-Gastric01 showed that the mOS of HER2-positive, IHC2+/FISH-, and IHC1+ were 12.6 months (95% CI: 0.4-33.2), 7.8 months (95% CI: 0.2-27.7) and 8.5 months(95% CI: 1.8-23.1) respectively, all meeting the primary endpoint of OS.

TABLE 1 Baseline characteristics of studies included in the systematic review with Bayesian network meta-analysis of third-line treatments for advanced gastric cancer.

Study (year)	Phase	Study Design	Sample size	Median age	Male/ Female	Intervention arm	Control arm	Tumor type	Reported outcomes
ATTRACTION-2 (22)	III	RCT	330/163	62/61	348/145	Intravenous infusion of nivolumab every 2 weeks for 6 weeks (one treatment cycle) (3 mg/kg)	Intravenous infusion of placebo every 2 weeks for 6 weeks (one treatment cycle) (3 mg/kg)	GC/ GEJC	OS,PFS, ORR,DCR, AE
Jin Li (10)	III	RCT	176/91	58/58	201/66	Oral apatinib 850 mg in tablet form once daily	Oral apatinib 850 mg in apatinib matching placebo once daily	GC/ GEJC	OS,PFS, ORR,DCR, AE
Jin Li (23)	II	RCT	47/48	55/65	75/20	Oral apatinib 850 mg once daily	Oral placebo 850 mg once daily	GC/ GEJC	OS,PFS, ORR,DCR, AE
DESTINY-Gastric01 (12)	II	RCT	125/62	65/66	147/45	Intravenous infusion of trastuzumab deruxtecan at a dose of 6.4 mg per kilogram of body weight every 3 weeks	Intravenous infusion of irinotecan monotherapy at a dose of 150 mg per square meter of body-surface area administered every 2 weeks or paclitaxel monotherapy, at a dose of 80 mg per square meter administered on days 1, 8, and 15 every 4 weeks.	GC	OS,PFS, ORR,DCR, AE
Kohei Shitara (11)	III	RCT	337/170	64/63	369/138	Oral trifluridine/ tipiracil (35 mg/ m^2 twice daily on days 1–5 and days 8–12 every 28 days) plus best supportive care	Oral placebo plus best supportive care	GC/ GEJC	OS,PFS, ORR,DCR, AE
Zhi Peng (15)	II	Non-RCT	125	58	91/34	RC48 2.5 mg/kg Q14d	/	GC/ GEJC	OS,PFS, ORR,DCR, AE

NMA of histopathology subgroup

For GC patients with different histopathology, nivolumab (HR: 0.62, 95% CI: 0.44-0.87), DS-8201 (HR: 0.38, 95% CI: 0.2-0.72), and chemotherapy (HR: 0.58, 95% CI: 0.39-0.86) all effectively prolonged the OS of patients with the intestinal type of GC over placebo, but no significant differences among these treatments were found. For patients with the diffuse type of GC, the OS of chemotherapy (HR: 2.63, 95% CI: 1.18-5.9) and placebo (HR: 3.82, 95% CI: 1.36-10.81) were significantly shorter than that of DS-8201. However, nivolumab did not achieve OS benefits over chemotherapy and placebo (Supplementary Figure 5A). The SUCRA values of DS-8201 was the largest for both patients with the intestinal type of GC (0.95) and patients with the diffuse type of GC (0.98) (Supplementary Figure 1).

NMA of previous gastrectomy, primary sites subgroup

For patients with a gastrectomy history, nivolumab (HR: 0.61, 95% CI: 0.47-0.78), DS-8201 (HR: 0.09, 95% CI: 0.03-0.3), and chemotherapy (HR: 0.57, 95% CI: 0.41-0.79) were superior to placebo. Furthermore, DS-8201 significantly prolonged the OS of patients compared with chemotherapy (HR: 0.16, 95% CI: 0.05-0.49) and nivolumab (HR: 0.15, 95% CI: 0.05-0.5). However, only nivolumab (HR: 0.71, 95% CI: 0.51-0.99) achieved OS benefits for patients in the absence of gastrectomy compared with placebo (Supplementary Figure 5B). Nivolumab (HR: 0.69, 95% CI: 0.56-0.86), DS-8201 (HR: 0.4, 95% CI: 0.24-0.66), and chemotherapy (HR: 0.67, 95% CI: 0.52-0.86) were superior to placebo in patients with gastric originated cancer. DS-8201 treatment showed significant OS benefits compared with chemotherapy (HR: 0.59, 95% CI: 0.38-0.92). However, only nivolumab (HR: 0.42, 95% CI: 0.2-0.89) prolonged the OS of patients with GEJC over placebo (Supplementary Figure 5C).

NMA of age, gender, ECOG and region subgroup

Nivolumab (HR: 0.6, 95% CI: 0.44-0.82) and DS-8201 (HR: 0.32, 95% CI: 0.17-0.61) significantly prolonged the OS of patients over 65 years old comparing with placebo. In addition, DS-8201 (HR: 0.44,

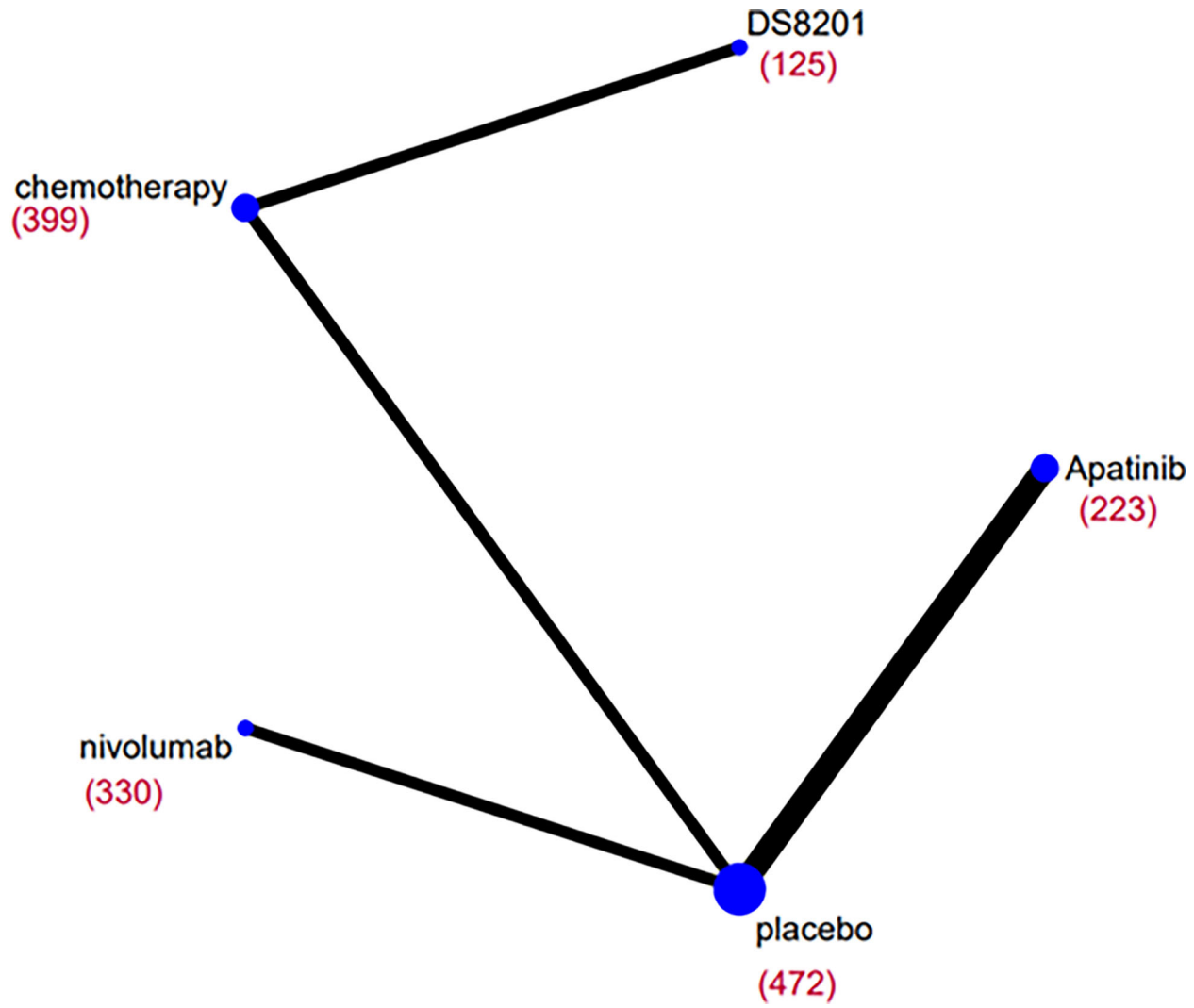

FIGURE 2
Network diagrams of comparisons on overall survival (OS) of treatments included in the network meta-analysis of the third-line treatments for advanced GC/GEJC. Each circular node represents a type of treatment. The size of the nodes and the thickness of the lines are weighted according to the number of studies evaluating each treatment and direct comparison, respectively. The total number of patients receiving treatments is shown in brackets.

95% CI: 0.26-0.75) was superior to chemotherapy. For patients less than 65 years old, nivolumab (HR: 0.7, 95% CI: 0.54-0.91) and chemotherapy (HR: 0.67, 95% CI: 0.51-0.89) achieved OS benefits over placebo (Supplementary Figure 5D). For males, the OS of nivolumab (HR: 0.6, 95% CI: 0.48-0.76), DS-8201 (HR: 0.34, 95% CI: 0.2-0.6), and chemotherapy (HR 0.65, 95% CI 0.5-0.84) were longer than that of placebo. However, DS-8201 was still superior to chemotherapy (HR: 0.53, 95% CI: 0.33-0.86). No third-line treatment had a significant effect on female patients (Supplementary Figure 5E). Nivolumab (HR: 0.62, 95% CI: 0.43-0.89), DS-8201(HR 0.38, 95% CI 0.19-0.75), and chemotherapy (HR 0.67, 95% CI 0.47-0.96) prolonged the OS of patients with ECOG PS=0 compared with that of placebo, which was the same as for patients with ECOG PS=1. Furthermore, DS-8201 can effectively improve the OS of patients over apatinib (HR: 0.49, 95% CI: 0.24-0.99) (Supplementary Figure 5F). The SUCRA values of DS-8201 was the largest for both patients with ECOG PS=0 (0.9) and patients with ECOG PS=1 (0.97). The results of subgroup analysis on Asian patients showed that nivolumab (HR: 0.46, 95% CI: 0.23-0.92), apatinib (HR: 0.71, 95% CI: 0.54-0.94), and DS-8201 (HR: 0.44, 95% CI: 0.22-0.86) prolonged the OS of patients significantly over placebo. However, DS-8201 still showed significant OS benefits over chemotherapy (HR: 0.57, 95% CI: 0.36-0.89) (Supplementary Figure 5G).

NMA of number of previous regimen treatments and metastasis sites subgroup

For patients with two previous lines of treatment, apatinib (HR: 0.7, 95% CI: 0.49-0.99) and chemotherapy (HR: 0.68, 95% CI: 0.47-0.97) were superior to placebo. Notably, DS-8201 improved the overall survival (OS) of patients with three previous lines of treatment compared with nivolumab (HR: 0.37, 95% CI: 0.15-0.9), apatinib (HR: 0.38, 95% CI: 0.14-0.99), chemotherapy (HR: 0.39, 95% CI:

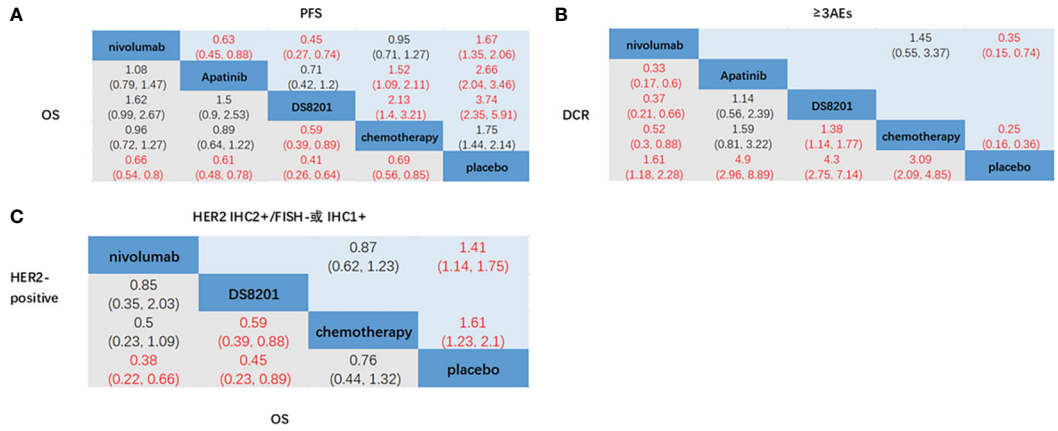

FIGURE 3
Network meta-analysis of the third-line treatments for advanced GC/GEJC. **(A)** Pooled hazard ratio (HR) [95% CrIs (credible intervals)] for overall survival (OS) and progression-free survival (PFS) in the overall population. **(B)** Pooled relative risk (RR) (95% CrIs) for disease control rate (DCR) and adverse events of grade 3 or higher (≥3AEs) in the overall population. **(C)** Pooled HR (95% CrI) for OS of patients with HER2-positive and HER2 IHC2+/FISH- or IHC1+.

0.18-0.85), and placebo (HR: 0.29, 95% CI: 0.12-0.68), while apatinib failed to achieve survival benefits (Supplementary Figure 5H). For patients with two or less metastasis sites, apatinib (HR: 0.7, 95% CI: 0.51-0.97), DS-8201 (HR: 0.27, 95% CI: 0.09-0.88) and chemotherapy (HR: 0.68, 95% CI: 0.49-0.95) were superior comparing with placebo. However, more than two metastasis sites were observed and apatinib failed to improve the OS of patients. Additionally, DS-8201 was superior to chemotherapy (HR 0.61, 95% CI 0.39-0.95) (Supplementary Figure 5I).

Rank probabilities

Bayesian ranking curves for various treatment options in different subgroups of patients are shown in (Supplementary Figures 1, 2). The results of Bayesian ranking were consistent with NMA approximately. For the overall population, ADC ranked first in both OS (0.98) and PFS (0.97), followed by apatinib and nivolumab. Apatinib ranked first in DCR (0.89). In addition, ADC ranked first in patients with a HER2-positive diagnosis, intestinal/diffuse histopathology, with or without previous gastrectomy history, gastric origination cancer, and ECOG PS=0/1, as well as in patient subgroups with two or more previous regimens, any numbers of metastasis site, and Asian patients. ADC was followed by nivolumab and apatinib. For patients with GEJC, nivolumab ranked first.

Model convergence, assessment of risk of bias, analysis of heterogeneity and inconsistency

As shown in Supplementary Figure 6, the results of the risk of bias assessment indicated low risk of bias for most RCTs and that non-RCTs were high-quality studies. The trace plots and Brooks-Gelman-Rubin diagnostics showed great convergence of the models we used (Supplementary Figure 7). In addition, the consistent model showed similar or better degree of fit than the inconsistent model in most of the comparisons (Supplementary Table 3). The heterogeneity between the available RCTs was large (I^2>50%) for primary outcomes. We performed a meta-analysis showing that JAVELIN Gastric300 (2018) (24) had a great influence on the heterogeneity of the NMA. No significant differences were found in the study design, median age of patients, or the ratio of male to female patients. In addition, factors such as age, sex, race, ECOG PS, primary sites, and PD-L1 expression status of patients in JAVELIN Gastric300 (2018) were analyzed in subgroups which showed that the statistical heterogeneity of each subgroup was low or medium (figure). Thus, we suggested that the heterogeneity between JAVELIN Gastric300 (2018) and other RCTs had little to do with the baseline characteristics of the patients. In addition, methodological heterogeneity was excluded because JAVELIN Gastric300 (2018) followed the principles of distribution concealment and blindness. Furthermore, the results of JAVELIN Gastric 300 suggested that the third-line treatment of GC/GEJC patients using the single drug avelumab did not lead to an improvement in OS or PFS over chemotherapy. Therefore, we excluded this RCT. In total, we included five RCTs with low statistical heterogeneity (I^2<50%) (Supplementary Figure 8).

Discussion

Combination therapy with anti-tumor drugs can prolong the OS of patients with GC/GEJC and lead to the improvement of patients' quality of life. Presently, third or later-line treatment options for advanced GC patients recommended by CSCO guidelines include immunotherapy (nivolumab), chemotherapy (irinotecan, FTD/TPI) and targeted therapy(apatinib) (7). For HER2-positive (IHC3+ or IHC2+/FISH+) patients, the guidelines recommended the use of ADC (trastuzumab deruxtecan, DS-8201) and virtuximab (RC48-ADC). However, in the phase III

ATTRACTION-2 trial, regardless of HER2 expression status, nivolumab significantly prolonged the OS of unresectable advanced or recurrent GC/GEJC (5.3 months vs 4.1 months) and reduced the risk of death (HR: 0.62, 95% CI: 0.50-0.75) (22) over placebo. T-DXd/DS8201 not only achieved significant survival benefits in GC patients who were HER2-positive, but also showed clinical activity in patients who were HER2-negative (IHC1+) or had low HER2 expression(IHC2+/FISH-) in the exploratory subgroup analysis of DESTINY-Gastric01(2020) (12). The results showed that the mPFS and mOS of the low-expression group were 4.4 months (95% CI: 2.7-7.1) and 7.8 months (95% CI: 4.7 -NE) respectively. The mPFS and mOS were 2.8 months (95% CI: 1.5-4.3) and 8.5 months (95% CI: 4.3-10.9), respectively, for the HER2-positive group. The median DOR of the two groups were 4.2 months (95% CI: 1.2-10.5) and 2.8 months (95% CI: 0.7-14.9), respectively. To further accurately screen and optimize the third-line treatment options through systematic review and NMA, the efficacy and safety of published third-line treatments for advanced GC/GEJC were reviewed to provide guide in selecting the best third-line treatment, so as to maximize precise individualized treatment plans for advanced GC/GEJC.

As shown in ATTRACTION-2 (22), Jinli (2016) (10)and Kohei Shitara (2018) (11), the OS of nivolumab (HR: 0.66, 95% CI: 0.54-0.8), apatinib (HR: 0.61, 95% CI: 0.48-0.78), and chemotherapy(HR: 0.69, 95% CI: 0.56-0.85) met the expected endpoint comparing with placebo in the third-line treatment of advanced GC/GEJC, with a mOS of 5.26 months (95% CI: 4.6-6.37), 6.5 months (95% CI: 4.8-7.6), and 5.7 months (95% CI: 4.8-6.2) respectively. In addition, nivolumab (RR: 1.61, 95% CI: 1.18-2.28), apatinib (RR: 4.9, 95% CI: 2.96-8.89), and chemotherapy (RR: 3.09, 95% CI: 2.09-4.85)were all superior to placebo, with a DCR of 40.2%, 42.0%, and 44.1% respectively. With the advent of ADC (DS-8201/RC48), which had been approved for the third-line treatment of GC/GEJC (15), the results of DESTINY-Gastric01 (2020) (12)showed that DS-8201 significantly prolonged the OS of patients in comparison with chemotherapy (HR: 0.59, 95% CI: 0.39–0.88), with mOS of 12.5 months (95% CI: 9.6–14.3). The DCR of DS-8201 was 85.7%, which was significantly improved comparing with that of chemotherapy (RR: 4.3, 95% CI: 2.75-7.14). This shows how the third-line treatment options of advanced GC continue to develop. The result of statistical heterogeneity test showed that the heterogeneity among RCTs we included was low (I2 < 50%), thus they were comparable. We then performed a NMA to compare the survival benefits of each third-line treatment option which showed that the SUCRA values of nivolumab (0.49) and apatinib (0.63) were higher than that of chemotherapy (0.41) in the general population, indicating that nivolumab and apatinib were superior to chemotherapy. This was consistent with the NMA results of third-line treatments of advanced GC/GEJC conducted by Park et al (25) and Huang et al (26). On this basis, the results of our NMA showed that the SUCRA value of DS-8201 (0.98) was the largest in OS. Furthermore, the NMA results of DCR showed that the SUCRA values of DS-8201 (0.84) and apatinib (0.89) were significantly higher than that of chemotherapy (0.52). DESTINY-Gastric01 showed that the most common ≥3AEs were the decreased neutrophil count (in 51% of the patients in the trastuzumab DS-8201 group), anemia (in 38% of the patients) and decreased white-cell count (in 21% of the patients). One death in the trastuzumab DS8201 group was considered by the investigators to be related to therapy, due to pneumonia, so it was concluded that its safety was controllable. In brief, we suggest that ADC is the best third-line treatment for advanced GC for the general population, followed by apatinib.

The overexpression of HER2 has been identified as a predictive biomarker in advanced GC, including IHC2+ and IHC3+, in which IHC3+, IHC2+/FISH+ are HER2-positive, while IHC1+ is HER2-negative (6). In a randomized, multicenter, phase 3 ToGA trial of Trastuzumab for Gastric Cancer, all patients for potential enrollment were tested for HER2 expression by IHC and FISH. The results showed that the ratio of HER2-positive IHC2+/FISH- was 17.8% and 5.3% (27). The first-line treatment of HER2-positive advanced GC with a combination of Trastuzumab with chemotherapy exhibited a median OS of 13.8 months and had efficacy outcomes correlated with the level of HER2 expression. In recent years, ADC (DS-8201, RC48) has offered a remarkable option in the third-line treatment for advanced GC patients with HER2-overexpression. The results of exploratory cohort study of DS-8201 by DESTINY-Gastric01 showed that the mPFS and mOS of the HER2-positive group were 5.6 months (95% CI: 4.3-6.9) and 12.6 months (95% CI: 0.4-33.2) respectively, compared with 4.4 months (95% CI: 2.7-7.1) and 7.8 months (95% CI: 0.2-27.7) for the IHC2+/FISH- group respectively, and 2.8 months (95% CI: 1.5-4.3) and 8.5 months (95% CI: 1.8-8) for HER2-negetive group respectively. A single-arm phase II study (2021) (15) showed that the mPFS and mOS in the RC48 group were 4.1 months (95% CI: 3.7-4.9) and 7.9 months (95% CI: 6.7-9.9), respectively. In addition, the exploratory subgroup analysis of ATTRACTION-2 compared the efficacy of nivolumab for patients with different HER2 expression statuses, and the results showed that the OS of patients with prior trastuzumab use was significantly longer comparing with that of placebo (HR: 0.38 95% CI: 0.22–0.66) which met the expected endpoint of OS. The mPFS, mOS, and median DOR were 1.6 months (95%CI: 1.5-4), 8.3 months (95% CI: 5.3-11) and 8.6 months (95% CI: 4.3-13.1) respectively. Similarly, for patients without prior trastuzumab use, nivolumab achieved OS benefits comparing with placebo (HR: 0.71, 95% CI: 0.57–0.88), and the mPFS, mOS, and median DOR were 1.6 months (95% CI: 1.5-2.4), 4.8 months (95% CI: 4.1-6), and 9.5 months (95% CI: 2.8-22.9)respectively. This result indicates that nivolumab was effective as a third or later-line treatment for GC/GEJC regardless of prior trastuzumab use (28) or HER2 expression status, and that nivolumab could benefit the survival of GC/GEJC patients comparing with placebo. We compared the relative efficacy of ADC, nivolumab, and chemotherapy in patients with different HER2 expression statuses based on NMA, and the results showed that the SUCRA value of DS-8201 (0.85) and nivolumab (0.8) were significantly higher than that of chemotherapy (0.29) in HER2-positive patients. For patients with HER2 (IHC2+/FISH- or IHC1+), the SUCRA value of nivolumab (0.61) was still higher than that of chemotherapy (0.39). Since DESTINY-Gastric01(2020) did not record the HR value of OS in patients who were HER2-positive (IHC2+/FISH-) or HER2-negative (IHC1+), it was impossible to further compare the relative efficacy of DS-8201 with other treatments. We also examined the reason why ADC (DS-8201, RC48) was effective as a third-line treatment for GC patients with HER2 overexpression. A derivative of DX-8951f (DXd), a topoisomerase I inhibitor, is coupled to the anti-HER2 antibody *via* a

peptide (GGFG) linker. This stable linker is cleaved upon internalization by lysosomal enzymes such as cathepsin B and L, which are highly expressed in tumor cells (29–31). As a result, ADC can be internalized into tumor cells *via* the HER2 receptor and cleaved by lysosomal enzymes, releasing DXd to specifically attack target molecules in tumor cells (32) which might be particularly effective in the treatment of tumors with overexpression or heterogeneous expression of HER2 (12).

In addition, our study also completed NMA of other subgroups, based on different demographic characteristics and pathological types. The results showed that for patients with intestinal type of GC, the SUCRA values of DS-8201, nivolumab, and chemotherapy were 0.95, 0.5, and 0.54, respectively. For patients with a gastrectomy history, the values were 0.99, 0.46, and 0.54, respectively, and for patients with gastric origination cancer, the values were 0.99, 0.48, and 0.53 respectively. The SUCRA ranking of patients with an ECOG PS score of 0-1 was the same as above, indicating that DS-8201 was significantly superior to chemotherapy, and may be the best third-line treatment for these patient groups, followed by nivolumab. For patients with GEJC, the results of ATTRACTION−2 (22) showed that nivolumab (HR: 0.42, 95% CI: 0.2–0.89) prolonged the OS of patients over placebo. Our NMA results showed that the SUCRA value of nivolumab (0.83) was significantly higher than that of DS-8201(0.66) and chemotherapy (0.42), indicating that nivolumab was the best third-line treatment option for patients with GEJC, which agreed with the results of subgroup analysis by Huang et al (26). Previous studies have demonstrated that GC in Western populations tends to originate mainly from GEJ (33). The pathological type of GEJC that includes a portion of squamous cell carcinoma tends to respond better to immunotherapy compared with adenocarcinoma. These factors might produce the more beneficial outcome of OS for GEJC from nivolumab (26). Notably, the results of ATTRACTION−2 (22), Jinli (2016), Kohei Shitara (2018), and DESTINY-Gastric01 showed that nivolumab (HR: 1.26, 95% CI: 0.87–1.83), DS-8201 (HR: 1.55, 95% CI: 0.66-3.71), and chemotherapy (HR: 1.22, 95% CI: 0.79-1.88) did not significantly prolong the OS of female patients over placebo, indicating that no third-line treatment had a significant effect on female patients. On the one hand, among the RCTs we included, there was less percentage of female patients than that of male patients, so the sample size was smaller, which may be related to the higher incidence of GC in male population (34). On the other hand, some studies have found that the pathological types of poorly differentiated adenocarcinoma and signet ring cell carcinoma were more frequently observed female GC patients than in male GC patients, and that these pathological types of tumor cells respond rather poorly to anticancer therapy (35, 36), thus leading to this result. In brief, we suggest that ADC is the best third-line treatment for the overall population of GC, as well as for patient groups with HER2-overexpression, intestinal histopathology, previous gastrectomy history, gastric origination cancer, and ECOG PS=0/1, followed by nivolumab and apatinib. However, nivolumab is the best third-line treatment for patients with GEJC.

The present study had the following limitations. Firstly, because of the limited number of clinical trials of third or later-line treatments for patients with GC/GEJC, the number of studies and patients we included were limited. As a result, the conclusions of NMA need to be further verified. Secondly, the node analysis using the Bayesian method or the direct element analysis using the frequency method was not carried out, because a closed loop in our NMA could not be established. Therefore, we cannot evaluate the analysis inconsistency caused by heterogeneity (37). In addition, the results of SUCRA ranking did not directly reflect the superiority of treatment regimens, and when SUCRA predictions were inconsistent with NMA results, we preferentially made judgments based on the HR of NMA and its 95% CI. For the original study took GC and GEJC patients as a whole and did not further compare the efficacy of the drugs on GC and GEJC patients separately, we were not yet able to further compare the relative efficacy and safety of several therapeutic drugs on GC and GEJC separately and hope to conduct future clinical trials on GC or GEJC patients separately. In order to verify the reliability of the NMA conclusion, we hope to carry out more multi-center real-world research on third-line therapies of advanced GC/GEJC patients in the future and compare the relative efficacy of different intervention methods to provide a guide for the formulation of precise individualized treatment plans. In addition, we hope that future RCTs will further study tumor progression, tumor markers and clinical symptoms of adverse reactions so that the efficacy and safety of drugs can be more comprehensively evaluated.

Conclusions

In summary, nivolumab, apatinib, chemotherapy, and ADC all improved the OS of GC/GEJC patients significantly. ADC may be the best third-line treatment option for the overall population of GC, as well as for patients with HER2-overexpression, intestinal histopathology, previous gastrectomy history, gastric origination cancer, ages over 65 and ECOG PS=0/1, followed by nivolumab and apatinib. Nivolumab may be the first treatment option for GEJC patients. For the limited clinical trials of third or later-line treatments for patients with GC/GEJC, these results need to be further confirmed by more multi-center real-world research in the future.

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

DY contributed to the study concept and design. YX and CZ participated in the initial literature search and evaluated the feasibility study for eligibility. YX and CZ interpreted the findings and wrote the first draft of the manuscript, prepared the figures and tables. JW contributed to language polishing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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Supplementary material

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Construction of a prognostic model for HCC based on ferroptosis-related lncRNAs expression and its potential to predict the response and irAEs of immunotherapy

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Background: Ferroptosis is an iron-dependent programmed cell death process, and studies have confirmed that it plays an important regulatory role in the occurrence and development of various malignancies including hepatocellular carcinoma (HCC). In addition, the role of abnormally expressed long non-coding RNAs (lncRNAs) in regulating and driving the occurrence and development of HCC has attracted more and more attention. However, there is still a lack of research on the role of ferroptosis-related lncRNAs in the prognosis prediction of HCC patients.

Method: In this study, we used the Pearson test method to analyze the association between differentially expressed lncRNAs and ferroptosis-related genes in HCC and normal tissues obtained from The Cancer Genome Atlas (TCGA), and found 68 aberrantly expressed and prognosis-related ferroptosis-related lncRNAs. Based on this, we established an HCC prognostic model composed of 12 ferroptosis-related lncRNAs. In addition, HCC patients were divided into a high-risk group and a low-risk group according to the risk score of this 12 ferroptosis-related lncRNAs prognostic model. Gene enrichment analysis indicated that ferroptosis-related lncRNA-based expression signatures may regulate HCC immune microenvironment signaling pathways through ferroptosis, chemical carcinogenesis-reactive oxygen species, and NK cell-mediated cytotoxicity pathways. In addition, immune cell correlation analysis showed that there were significant differences in immune infiltrating cell subtypes, such as Th cells, macrophages, monocytes, and Treg cells between the two groups. In addition, the expression of multiple immune checkpoint molecules was found to be significantly increased in the high-risk group (eg, PD1, CTLA-4, CD86, etc.).

Results: Our research provides a new method for predicting prognosis using a ferroptosis-related lncRNA expression signature prognostic model in hepatocellular carcinoma. And it provides new tools for predicting patient response and adverse effects of immunotherapy.

Conclusion: In conclusion, ferroptosis-related lncRNA expression signatures can be used to construct a prognostic prediction model to predict the overall survival

of HCC patients, and can be used as an independent influencing factor for prognosis. Further analysis showed that ferroptosis-related lncRNAs may affect the efficacy of immunotherapy in patients with HCC by altering the tumor microenvironment, so this model may serve as a new indicator of the response and irAEs of HCC to immunotherapy.

KEYWORDS

ferroptosis, immune checkpoint blockers, immune-related adverse events, lncRNA, heptocellular carcinoma

1 Background

Hepatocellular carcinoma (HCC) is the second most common cause of death from human malignancies worldwide and the most common liver malignancy. Llovet et al. (2016) According to research statistics, about 841,000 new cases of hepatocellular carcinoma are diagnosed each year, and about 780,000 patients will die from hepatocellular carcinoma in 2018. Bray et al. (2018) For patients with early-stage hepatocellular carcinoma, local radiofrequency ablation, partial hepatectomy, and liver transplantation are the main treatments, but about 70% of patients will suffer a recurrence within 5 years after surgery. Bray et al. (2018) In recent years, immune checkpoint inhibitors have been proven by many studies to be an effective therapy for the treatment of advanced hepatocellular carcinoma, but their effectiveness still needs to be further improved. Ozer et al. (2021) Although great progress has been made in the early detection and drug treatment of hepatocellular carcinoma, the clinical outcomes of advanced cases are still unsatisfactory. The SEER database shows that the overall 5-year survival rate for hepatocellular carcinoma patients in the United States is 19.6%, while the 5-year survival rate for patients with distant metastases is less than 2.5%. Chidambaranathan-Reghupaty et al. (2021) Due to the high heterogeneity of HCC, there is an urgent need to find new effective molecular markers and improve the prediction accuracy of HCC prognosis to improve the clinical outcomes of HCC and reduce the burden on patients.

Cell death is an essential part of many important physiological and pathological processes in the human body. Vermeulen et al. (2003) Ferroptosis is a relatively new programmed cell death process newly discovered in recent years. It is distinct from other cell death processes such as necrosis, apoptosis, and autophagy. Dixon et al. (2012) Ferroptosis is a form of iron-dependent programmed cell death caused by the accumulation of reactive oxygen species (ROS) generated by lipid peroxidation in cells. Recently, the induction of ferroptosis in tumor cells has become a promising new therapy in the eyes of researchers, especially for malignant tumors that are resistant to conventional radiotherapy and chemotherapy (Liang et al., 2019; Xia et al., 2019; Du et al., 2021; Bekric et al., 2022). With the recent FDA approval of anti-PD-1 or anti-PD-L1 drugs (Keytruda, Tecentriq, nivolumab), immune checkpoint blocker (ICBs) therapy as a new therapy for patients with advanced HCC has gained more and more attention from researchers. Various immune checkpoint inhibitors, alone or in combination with targeted therapy and traditional chemotherapy, are also increasingly used to treat patients with advanced hepatocellular carcinoma (Pinter et al., 2021a; Pinter et al., 2021b; Wu et al., 2022). However, only part of these patients can benefit from immunotherapy, possibly due to the complexity and heterogeneity of the tumor itself, as well as many unknown factors in the tumor microenvironment (TME) (El Dika et al., 2019; Lee et al., 2020; Zhu et al., 2022). The complex tumor microenvironment may reduce the efficacy of immunotherapy, and the underlying mechanism may be related to various stromal cells and various types of immunosuppressive factors contained in the microenvironment. Prieto et al. (2015) Therefore, it is crucial to further explore novel molecular mechanisms in HCC and develop a new indicator to evaluate the response of HCC patients to immunotherapy, thereby optimizing the treatment strategy. A recent study found that $CD8^+$ T-cells induced by immunotherapy could enhance ferroptosis by altering the microenvironment and releasing cytokines, thereby reducing the expression level of SLC7A11 in tumor cells to suppress the tumor Wang et al. (2019). This suggests a relationship between the ferroptosis process in tumor cells and immune system activation. Another study has shown that tumor cells with ferroptosis may act as donor cells to produce biologically active immunomodulatory arachidonic acid metabolites to affect anti-tumor immunity Friedmann Angeli et al. (2019). Therefore, it is necessary to study tumor immunotherapy from the perspective of the ferroptosis mechanism. A large number of experimental studies have also shown that ferroptosis-related genes play a crucial role in the occurrence and development of hepatocellular carcinoma (Sun et al., 2016; Liao et al., 2021; Wang et al., 2021; Yao et al., 2021).

Long non-coding RNAs (lncRNAs) are self-transcribed non-coding RNAs with a minimum fragment length of about 200 nucleotides, which can participate in various complex biological processes (Cech and Steitz, 2014; Quinn and Chang, 2016). Previous studies have shown that lncRNAs are abnormally expressed in a variety of malignant tumors, and other studies have shown that abnormally expressed lncRNAs can be used as prognostic indicators for various malignancies including hepatocellular carcinoma (Xu et al., 2022a; Xu et al., 2022b; Cui et al., 2022; Zhou et al., 2022; Zhu et al., 2022). By interacting with proteins or DNAs, lncRNAs play important roles in the occurrence and progression of different types of tumors, including HCC Huang et al. (2020). However, studies on ferroptosis-related lncRNAs related to the prognosis of HCC patients are still insufficient. Therefore, this study aimed to establish a novel prognostic model of ferroptosis-related lncRNAs expression signature to predict the prognosis of HCC patients and to improve the current diagnosis, treatment, follow-up, and prevention of HCC.

In the present study, we identified the expression signatures of lncRNAs associated with ferroptosis in hepatocellular carcinoma by correlation analysis and constructed a new prognostic model based on 12 ferroptosis-associated lncRNAs using multivariate Cox regression analysis. Then we assessed the ability of this model to

independently predict the prognosis of HCC patients and explored the role of ferroptosis-related lncRNAs in tumor immunity. In conclusion, this study found that ferroptosis-related lncRNA can affect the efficacy of immunotherapy by affecting immune cell infiltration in the tumor microenvironment, so it has the potential to serve as an ideal biomarker for evaluating the therapeutic effect and adverse effects of immunotherapy.

2 Methods

2.1 Data and information collection

In this study, the transcriptome RNA sequencing (RNA-seq) data of 371 hepatocellular carcinoma patients with complete clinical data were downloaded from the TCGA official website (http://portal.gdc.cancer.gov/). This study normalized the mRNA expression data for each patient using an algorithm provided by the R package (Limma). The corresponding clinical and pathological characteristics of the enrolled patients, including age, gender, tumor differentiation, TNM stage, survival time, and survival status, were also downloaded from the TCGA database. The data involved in the TCGA database are publicly available, therefore, this study does not require ethics committee approval.

2.2 Identification of lncRNAs associated with the ferroptosis

The FerrDb database is the first manually organized ferroptosis database established by Chinese researchers. The database includes ferroptosis-related driver and suppressor genes, markers, various regulatory factors, and ferroptosis-related disease data. In this study, ferroptosis-related genes were retrieved from the FerrDb (http://www.zhounan.org/ferrdb/) database. A total of 382 ferroptosis-related genes were finally included. Relationships between lncRNAs and ferroptosis-related genes were calculated based on RNA expression levels. Co-expression analysis was performed using Spearman's correlation coefficient to identify lncRNAs related to ferroptosis. The absolute value > 0.4, and the p-value < 0.001 were defined as ferroptosis-related lncRNAs.

2.3 Construction and validation of ferroptosis-related lncRNA prognosis prediction model

Firstly, lncRNA expression and clinical data were analyzed. Ferroptosis-related lncRNAs associated with prognosis were identified using univariate Cox regression. Then, ferroptosis-related lncRNAs with p values ≤0.05 were included in multivariate Cox regression to construct a prognostic prediction model based on the expression of ferroptosis-related lncRNAs. The risk score formula used in the prognostic model is as follows: risk score = $e^{\text{sum (lncRNA expression} \times \text{corresponding coefficient)}}$. Patients were separated into low-risk or high-risk groups based on the median value. Differences in survival status between the two risk groups were assessed by Kaplan-Meier (KM) and tested with the log-rank test method. The ROC curve and calibration curve were used to determine the accuracy of the prognostic prediction model. Then, combined with other clinical characteristics of the enrolled patients, it was determined whether the prognostic prediction score could be used as an independent influencing factor of prognosis, and a nomogram was drawn below.

2.4 Functional enrichment analysis of related lncRNA genes

In this study, the R package (ClusterProfiler) was used to perform GO enrichment analysis (Gene Ontology, GO) and KEGG enrichment analysis (Kyoto Encyclopedia of Genes and Genomes, KEGG). p values were still adjusted by the BH method. Gene set enrichment analysis (GSEA) in the R package (gsva) was used to investigate functional phenotypic differences between two risk groups (high-risk group and low-risk group). In this study, we functionally enriched ferroptosis-related lncRNAs and visualized the pathways that are closely related to immunity and tumorigenesis and development. The gene sets used were downloaded from the Molecular Signatures database and analyses were run in GSEA software (version 4.2.3). p values <0.05 and FDR <0.05 were considered statistically significant.

In the statistical analysis of this study, all p values were two-tailed and $p < 0.05$ was considered statistically significant. Kaplan-Meier survival curves were used to compare survival differences between different risk groups (low-risk and high-risk groups). Univariate and multivariate Cox regression analyses were used to identify independent clinical prognostic factors. In the GSEA analysis comparing immune cells and immune-related functions between the two groups, the differences in scores were tested by the Mann-Whitney test. All statistical analyses were performed in R software (version 4.1.3). Relevant R packages used in the study include ggplot2, stats, Rtsne, timeROC, glmnet, gsva, survival, and survminer, etc.

3 Results

3.1 lncRNAs associated with ferroptosis in hepatocellular carcinoma

A list of 382 ferroptosis-related genes was first extracted and downloaded from the FerrDb database. And lncRNAs with significant correlation with ferroptosis genes were found through co-expression analysis. The filter condition was set to the correlation coefficient Cor>0.4, and the p-value < 0.001. After co-expression analysis, 1,278 lncRNAs related to ferroptosis were obtained. The obtained ferroptosis-related lncRNAs were further used in a univariate Cox proportional hazards regression model to find out which lncRNAs were associated with prognosis.

3.2 Construction and validation of ferroptosis-related lncRNA prognosis prediction model

Combined analysis of ferroptosis-related lncRNAs and survival data, using univariate Cox regression, the analysis showed that

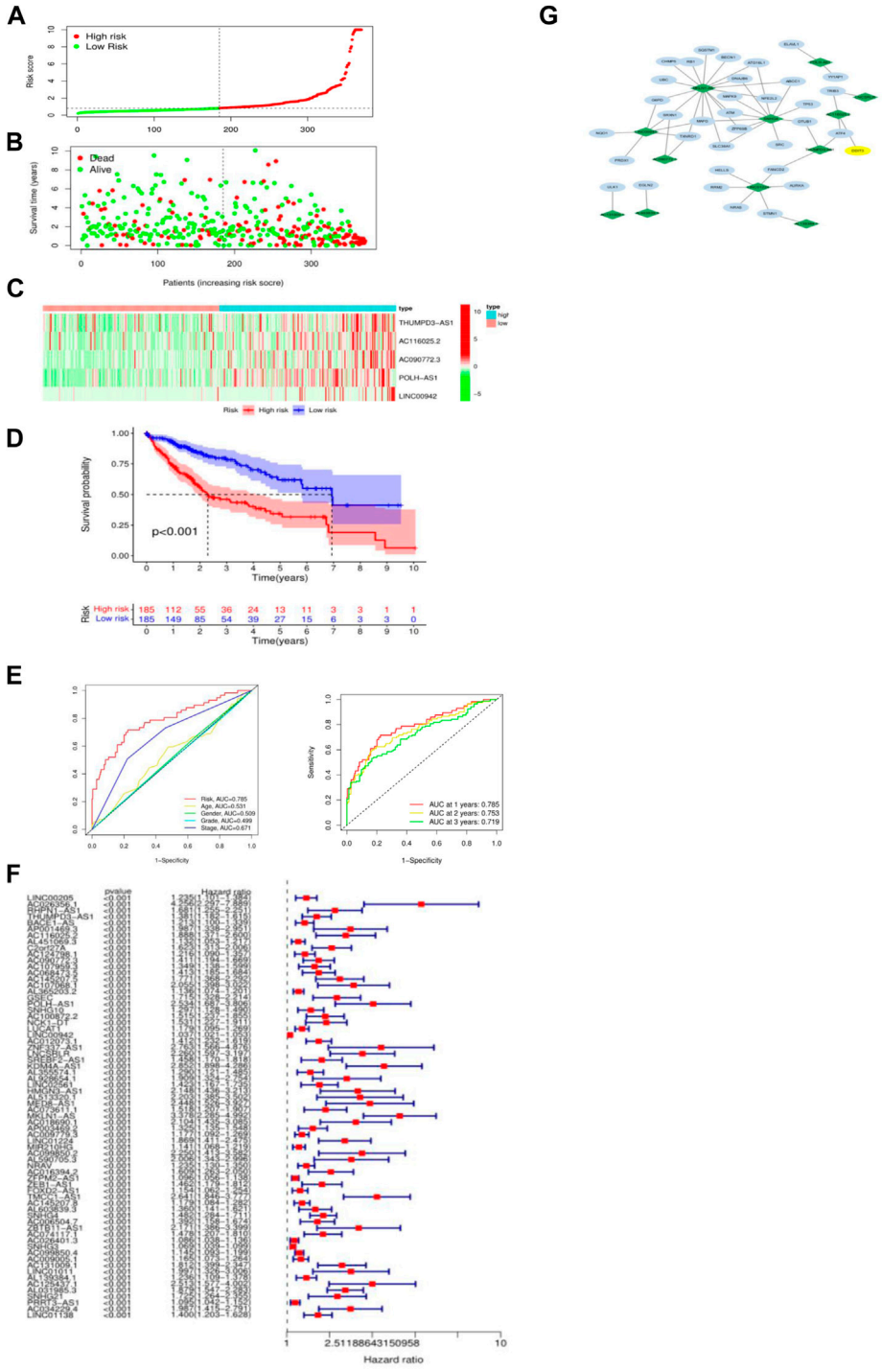

FIGURE 1
(A) Risk score distribution and median value in TCGA HCC cohort; **(B)** Survival status, overall survival time (OS), and risk score distribution of HCC patients in TCGA cohort; **(C)** Heatmap of expressions of 12 selected ferroptosis-related lncRNAs in high-risk and low-risk groups; **(D)** Kaplan-Meier survival curves of two groups of patients (high-risk group and low-risk group); **(E)** The AUC for risk score and clinical features according to the ROC curves, and ROC curve analysis within 1, 2, and 3 years. **(F)** Ferroptosis-related lncRNA expression and overall survival Forest plot of univariate Cox regression analysis of period (OS); **(G)** Schematic diagram of ferroptosis-related lncRNA and mRNA correlation network (diamond is lncRNA, oval is ferroptosis-related gene mRNA).

68 ferroptosis-related lncRNAs were closely related to overall survival (OS) in patients with hepatocellular carcinoma, and the high expressions of these lncRNAs were associated with poor prognosis. (Figure 1F). With LASSO Cox regression analysis, finally, 12 lncRNAs related to ferroptosis were screened, and based on their expression data, a prognostic prediction model for hepatocellular carcinoma patients was constructed. The calculation formula in the model is: Risk Score = 0.288 × THUMPD3-AS1 expression +0.538

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× AC116025.2 expression +00.201 × AC090772.3 expression +0.797 × POLH-AS1 expression +0.031 × LINC00942 expression +0.695 × LNCSRLR expression +0.785×MKLN1-AS expression +0.302×LINC01224 expression +0.277 × AL603839.3 expression +0.332×SNHG4 expression +0.411 × AC131009.1 expression +0.214 × AL139384.1 expression. The hazard ratio of each lncRNA to survival time (OS) in this model was greater than 1, and the expression in HCC tissue was significantly higher than that in normal liver tissue. Patients were classified into a high-risk group (n = 185) and a low-risk group (n = 185) according to the median TCGA group risk score (0.828). The distribution of risk scores of the two groups is shown in the figure (Figure 1A), and the distribution of survival status of patients also shows that the overall survival of patients in the high-risk group is significantly shortened in the lower-risk group (Figure 1B). The heatmap of lncRNA expression involved in the construction of the prognostic prediction model showed that all 12 ferroptosis-related lncRNAs were highly expressed in the high-risk group (Figure 1C). The Kaplan-Meier survival curves of the two groups of patients showed that the overall survival (OS) of patients in the high-risk group was significantly lower than that of the patients in the low-risk group ($p < 0.0001$) (Figure 1D).

The predictive performance of the model in predicting the overall survival (OS) risk score was then evaluated by time-dependent ROC curves, with AUC reaching 0.785 at 1 year, 0.753 at 2 years, and 0.719 at 3 years (Figure 1E). To further analyze the interaction between 12 ferroptosis-related lncRNAs and ferroptosis-related gene expression, Cytoscape software was used to visualize the co-expression network of lncRNAs and mRNAs. Death-related genes are at the center of the correlation network. (Figure 1G).

3.3 The independent prognostic value of this prediction model based on 12 ferroptosis-related lncRNA expression signatures

To further validate the prognostic value of this risk score model, we performed univariate and multivariate Cox regression analyses using patient age, sex, tumor grade, TNM stage, and risk score as variables. Results could determine whether risk score can be used as an independent prognostic predictor of overall survival (OS). In univariate Cox regression analysis, the risk score of the TCGA cohort was significantly associated with overall survival (OS) (HR = 1.227, 95% CI = 1.177–1.280, $p < 0.001$) (Figure 2A). After adjusting for other confounding factors, risk score remained an independent predictor of overall survival (OS) in multivariate Cox regression analysis (HR = 1.228, 95% CI = 1.172–1.287, $p < 0.001$) (Figure 2B). These results confirmed that this new HCC patient prognosis prediction model based on ferroptosis-related lncRNA expression signature can be reliably used as a novel tool for HCC patient prognosis prediction. To make the prognostic prediction model based on ferroptosis-related lncRNA more applicable to the clinic, this study also established a nomogram to better help clinicians to predict the 1-, 3-, and 5-year survival rates of patients. The predictors in the nomogram included the risk score and other clinicopathological characteristics (age, gender, tumor grade, tumor stage) of the predictive model (Figure 2C). In the plotted nomogram, the risk score model exerted excellent weights in all of these clinically relevant variables, which is also consistent with the results of the multivariate Cox regression analysis. These results collectively confirm that this novel lncRNA prediction model associated with ferroptosis can reliably serve as an independent prognostic factor in HCC patients.

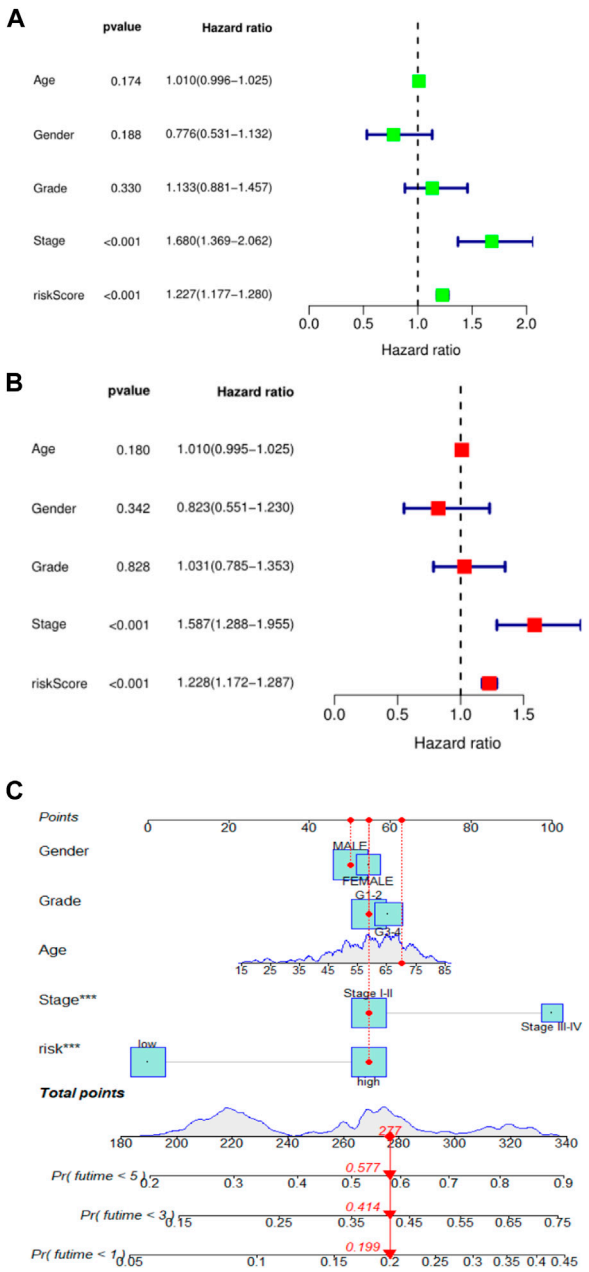

FIGURE 2
(A). Univariate Cox regression analysis of risk score and survival in TCGA cohort; **(B)**. Univariate Cox regression analysis of risk score and survival in TCGA cohort; **(C)**. nomogram of 1, 3, and 5-year survival rates of liver cancer patients.

3.4 Functional enrichment analysis of ferroptosis-related genes

To further understand the molecular mechanism of ferroptosis-related differentially expressed genes and how it affects the occurrence

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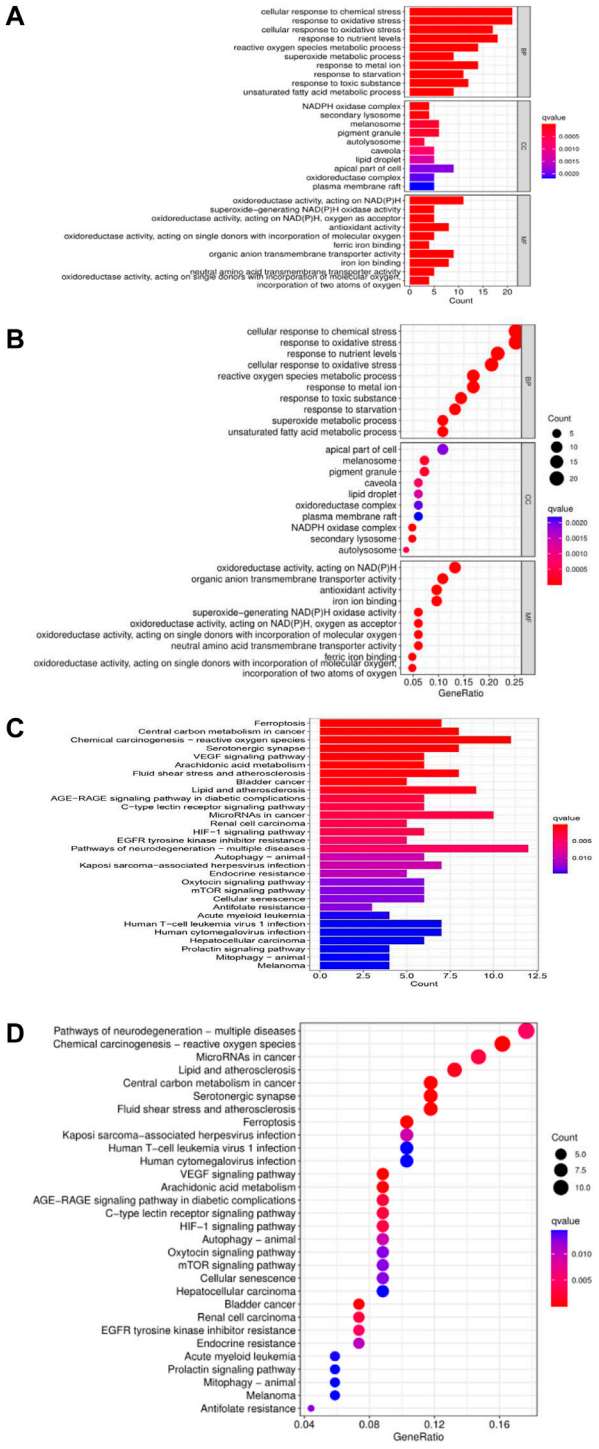

FIGURE 3
GO enrichment analysis of ferroptosis-related lncRNAs in the TCGA liver cancer cohort **(A, B)** and KEGG enrichment analysis **(C, D)**.

and development of hepatocellular carcinoma, GO enrichment and KEGG enrichment analysis were also performed in this study. GO enrichment analysis showed that: in the RNA-seq expression data of TCGA HCC patients, in terms of biological processes, it can be observed that related genes are enriched in various cellular stress response processes, such as cellular oxidative stress, cellular chemical stress, etc. Consistent with the expected results, there were significant enrichment phenomena in the intracellular redox reaction chain and iron metabolism, including a variety of enzymes involved in NADPH oxidation, antioxidant reaction processes, iron ion binding, transmembrane transporters, the redox reaction of molecular oxygen, etc. KEGG enrichment result was also as predicted before, these genes were enriched in ferroptosis, chemical carcinogenesis process - reactive oxygen species (ROS), superoxidation process, mTOR signaling pathway, and autophagy-related signaling pathway. And it is related to a variety of malignant tumor-related pathways, including acute myeloid leukemia-related pathways, renal cell carcinoma, and bladder cancer-related pathways. In addition, it can be observed that these genes are enriched in the EGFR tyrosine receptor signaling pathway, the VEGF receptor pathway, etc., which also implies that ferroptosis may play a certain role in the targeted therapy of hepatocellular carcinoma. (Figure 3).

To further explore the mechanism of ferroptosis-related lncRNA in the occurrence and development of hepatocellular carcinoma. We performed GSEA analysis, and the results showed that the enrichment of gene sets in high-risk group patients included cell adhesion pathway, apoptosis pathway, cell cycle pathway, DNA replication, endocytosis, fatty acid metabolism, insulin receptor pathway, and mTOR-like receptors. In addition, some immune-related pathways were also significantly enriched in the high-risk group, including B-cell receptor (BCR), T-cell receptor (TCR), NK cell-mediated cytotoxic effector pathways, etc. These results suggest that patients with high-risk scores in this predictive model may be associated with enhanced DNA replication, abnormal metabolic pathways, activation of some classical tumor signaling pathways, and tumor immune escape (Figure 4).

3.5 Relationship between ferroptosis-related lncRNAs and tumor-infiltrating cells in hepatocellular carcinoma

To further explore the mechanism of ferroptosis-related lncRNAs involved in the occurrence and development of hepatocellular carcinoma, we used the algorithms of CIBERSORT, CIBERSORT-ABS, XCELL, EPIC, MCPCOUNTER, QUANTISEQ and TIMER to draw a heat map of immune cell correlations as shown below. It was found that some immune-infiltrating cells, TICs, including dendritic cells, neutrophils, macrophages, mast cells, monocytes, and regulatory T (Treg) cells were enriched in the high-risk group significantly higher than in the low-risk group. These findings strongly suggest that our selected ferroptosis-related lncRNA expression signature is significantly associated with immune cell infiltration in HCC (Figure 5).

3.6 Correlation between ferroptosis-related lncRNA signatures and ICB treatment outcomes and irAEs

Previous sections suggested a correlation between ferroptosis-related lncRNAs, tumor-infiltrating cells, and immunological signatures. Based on these findings, we further investigated the role of these lncRNAs in immunotherapy treatment and adverse

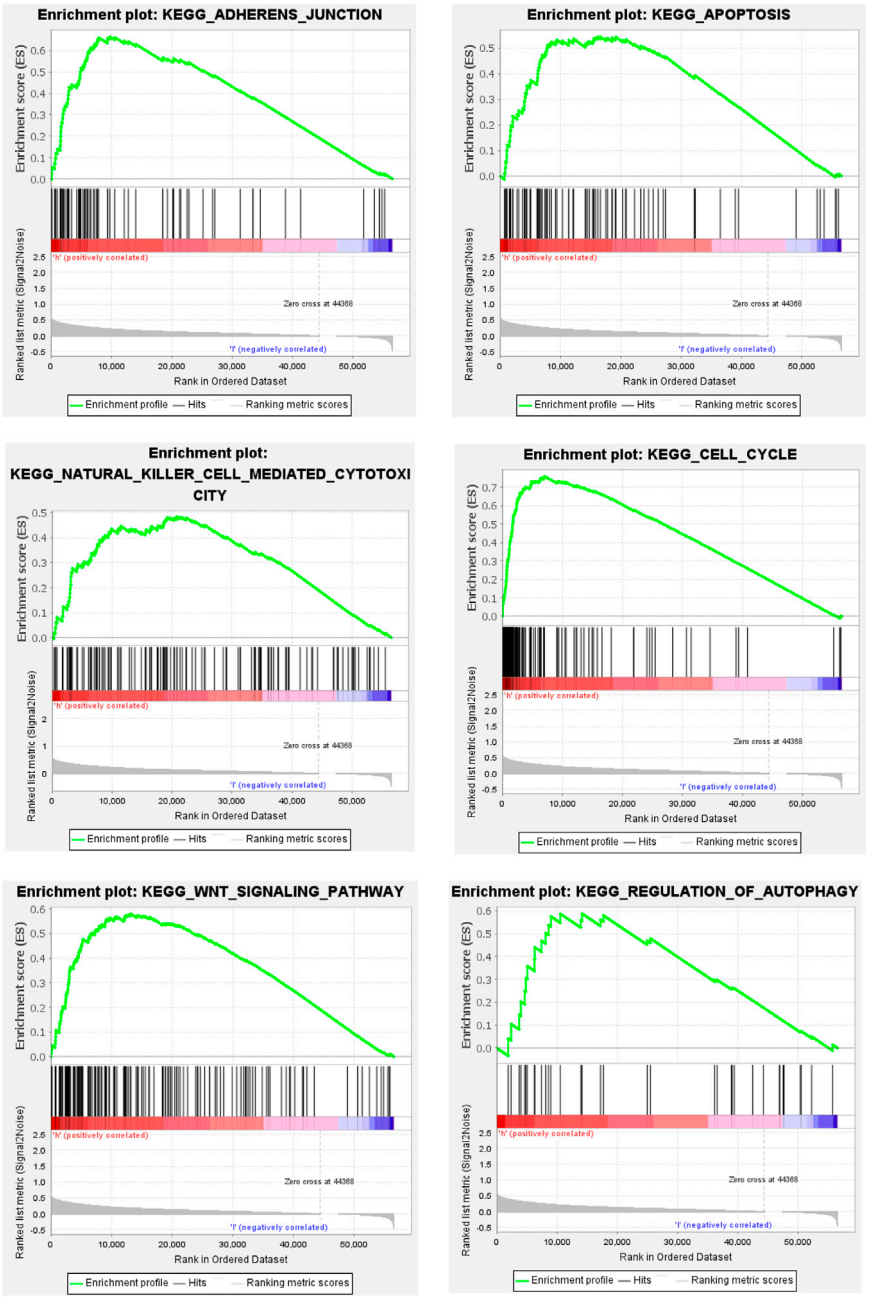

FIGURE 4
Partial GSEA enrichment analysis results.

effects. We found that the expression levels of the above ferroptosis-related lncRNAs were significantly correlated with immune checkpoint gene expression (PD-1 (p-value <0.05), CTLA-4 (p-value <0.05), IDO2 (p-value <0.05), CD44 (p-value <0.05), LAG3 (p-value <0.05)). This suggests that abnormally high expression of immune checkpoint proteins can be observed in patients in the high-risk group. Some of these proteins were also identified as an independent predictor of irAEs (immune-related adverse events) development. This suggests that patients in the high-risk group may benefit from immunotherapy and have a greater chance of developing irAEs (Figure 6).

4 Discussion

Hepatocellular carcinoma is one of the most common malignant tumors in the world with a high mortality rate. Due to the heterogeneity of the tumor itself, it is extremely difficult for clinicians to predict the prognosis of patients. Therefore, it is very important to develop a reliable and effective prognostic biomarker for HCC. In this study, we developed a novel prognostic model based on 12 ferroptosis-related lncRNA expression signatures in the TCGA HCC cohort, and it shows good prediction performance.

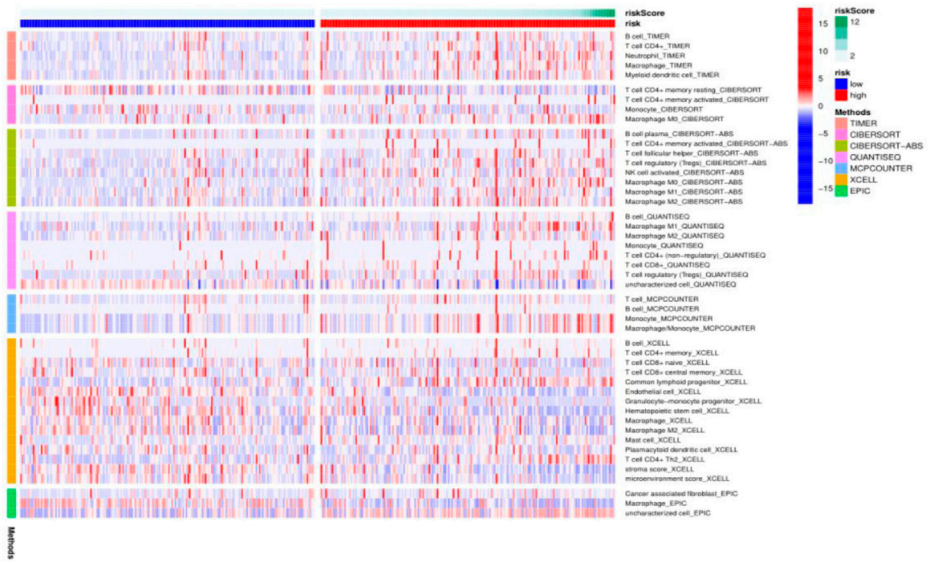

FIGURE 5
Heat map of immune cell correlation analysis in TCGA HCC cohort ferroptosis-related lncRNA prognostic model.

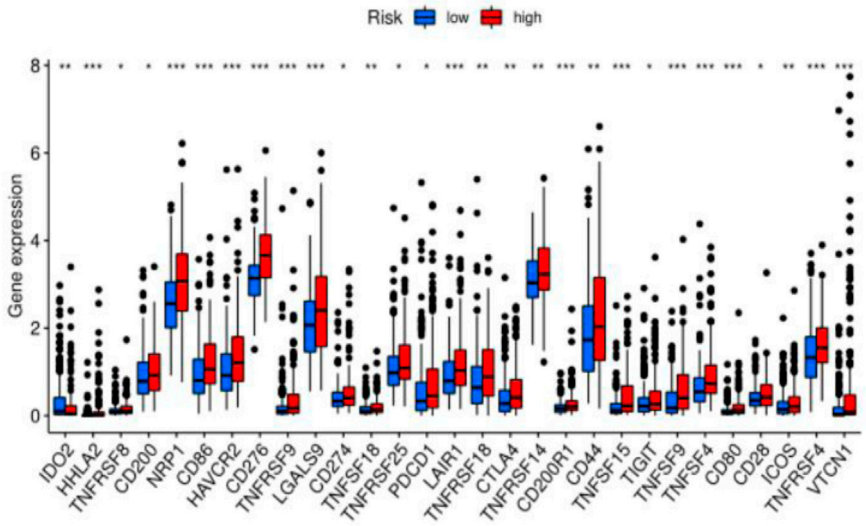

FIGURE 6
Difference analysis of immune checkpoints between two groups (high-risk group and low-risk group) in the TCGA cohort.

Studies have shown that lncRNAs play a key role in the chromatin structure, cell growth, gene expression, differentiation, and development of human cells, and their abnormal expression or mutation is closely related to a variety of diseases, especially malignant tumors (Peng et al., 2017; Sato et al., 2021; Wu et al., 2021). It is believed that lncRNAs are associated with multiple malignant tumor-related processes, such as proliferation, invasion, migration, and angiogenesis (Schmitt and Chang, 2016). For the treatment of hepatocellular carcinoma, lncRNAs can be used as biomarkers to predict the efficacy of patients receiving surgery, radiotherapy, chemotherapy, and immunotherapy, and it is expected to become an important tool for individualized diagnosis and treatment of hepatocellular carcinoma. Yuan et al. (2021) In existing studies, many scholars have used a variety of lncRNA expression features to predict the prognosis of various malignant tumors and constructed different prognostic models, including breast cancer, colorectal cancer, lung cancer, gastric cancer, bladder cancer, etc. (Liu et al., 2020; Ma et al., 2020; Shen et al., 2020; Song et al., 2021; Xu et al., 2021). In HCC, other researchers have developed a variety of lncRNA expression signature-based prognostic prediction models based on differentially expressed lncRNAs and certain tumor pathogenesis. For example, the 11 lncRNAs (AC010547.1, AC010280.2, AC015712.7, GACAT3, AC079466.1, AC089983.1, AC051618.1, AL121721.1, LINC01747, LINC01517, and

AC008750.3) expression signatures can be used to effectively predict the risk of death from hepatocellular carcinoma (Li et al., 2020). Another study expression signatures also constructed a liver cancer prognosis model using seven autophagy-related lncRNAs (PRRT3-AS1, RP11-479G22.8, RP11-73M18.8, LINC01138, CTD-2510F5.4, CTC-297N7.9, RP11-324I22.4) and demonstrated good predictive performance (Yang et al., 2021). In addition, the biological functions of selected lncRNAs in hepatocellular carcinoma have been confirmed in multiple independent studies, for example, MKLN1-AS can affect HCC epithelial-mesenchymal transition (EMT) through the SOX9-MKLN1-AS axis, which promoted the proliferation and migration of hepatocellular carcinoma cells (Guo et al., 2022). LINC01224 could downregulate the expression of CHEK1 through competitive binding with miR-330-5p, thereby inhibiting the progression of hepatocellular carcinoma (Gong et al., 2020).

With the development of immune checkpoint inhibitors, immunotherapy as an emerging therapy has shown a considerable therapeutic effect on hepatocellular carcinoma (Foerster et al., 2022; Llovet et al., 2022). Currently, immunotherapy combined with anti-angiogenic targeted therapy provides a new promising treatment strategy for advanced liver cancer. However, more than two-thirds of patients still show an unsatisfied response to immunotherapy (Mushtaq et al., 2018). A recent study showed that ferroptosis combined with immune checkpoint inhibitors can synergistically enhance antitumor activity, a phenomenon seen even in immunotherapy-resistant tumors (Tang et al., 2020). Therefore, a new predictive model based on the expression characteristics of ferroptosis-related lncRNAs can be considered to study the relationship between immunotherapy and ferroptosis and predict the efficacy of immunotherapy. In our study, we found that the expression signature of the lncRNAs we selected was related to the expression of immune checkpoint proteins (i.e., PD-1, CTLA-4 and CD28, etc.). This suggests that the model could potentially be used to predict patients' responses to immunotherapy. Meanwhile, the expression levels of these immune checkpoint proteins were higher in the high-risk group than in the low-risk group. This indicates that the expression signature of ferroptosis-related lncRNAs can be used to predict the expression level of immune checkpoint proteins in tumor tissues, and has the potential to be seen as a new indicator to guide immunotherapy decisions. With the wide application of ICBs in the treatment of HCC, the toxic and side effects caused by the activation of the immune system by ICI, which is, immune-related adverse events, have become a major challenge in clinical practice (Postow et al., 2018). There are no validated biomarkers to predict the irAEs before ICBs treatment until now. Some genes are associated with irAEs, and the expression levels of these genes were higher in the high-risk group in our study. It suggests that our model has the potential to predict the occurrence of irAEs. But these findings still need to be proved in larger studies, and multi-omics prediction could have better performance (Jing et al., 2020; Wölffer et al., 2022). In addition, this study also showed that the risk score of ferroptosis-related lncRNAs expression signature was associated with immune infiltrating cells (B-cells, macrophages, myeloid dendritic cells, neutrophils, and $CD4^+$ T-cells) in HCC tissues, which means that this prognostic model may play an important role in immune infiltration.

However, our study still has some limitations. This study is primarily a retrospective study based on comprehensive bioinformatics analysis and public database data, and these findings lack solid clinical validation. In addition, the accuracy of the ferroptosis-related lncRNA expression signature prognostic model for the immune regulation of HCC patients will remain an important clinical issue, which needs to be verified by prospective experiments.

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

In this research, XH made significant contributions to research conception, design of study, and revised the manuscript. LD collected, analyzed the data and wrote the manuscript. SZ and XB provided critical revisions. All authors agree to be accountable for all aspects of the work, and questions relevant to accuracy or integrity are dealt with and surveyed in an appropriate way. The final manuscript has been read and approved by all authors.

Conflict of interest

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

Publisher's note

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Pneumatosis intestinalis post steroid use in a patient with immune-related adverse events: Case report, literature review and FAERS analysis

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Introduction: The accurate diagnosis of pneumatosis intestinalis (PI) is increasing despite patients' limited identification of etiologic factors. Recently a patient with lung squamous carcinoma who developed pneumatosis intestinalis following methylprednisolone administration for immune-related adverse events was treated at our hospital. Subsequent a literature review and an analysis of the FDA Adverse Event Reporting System (FAERS) database enabled the identification of additional cases of pneumatosis intestinalis.

Methods: A literature review of the MEDLINE/PubMed and Web of Science Core Collection databases using standard pneumatosis intestinalis search terms to identify published cases of immune checkpoint inhibitors (ICIs) or steroids causing pneumatosis intestinalis were performed. A separate retrospective pharmacovigilance study of FAERS enabled the extraction of unpublished cases of pneumatosis intestinalis between the first quarter of 2005 and the third quarter of 2022. Disproportionality and Bayesian analyses were performed to identify signal detection in reported odds ratios, proportional reporting ratios, information components, and empirical Bayesian geometric means.

Results: Ten case reports of steroid-related pneumatosis intestinalis were retrieved from six published studies. The implicated drug therapies included pre-treatment with steroids before chemotherapy, combination therapy with cytotoxic agents and steroids, and monotherapy with steroids. In the FAERS pharmacovigilance study, 1,272 cases of immune checkpoint inhibitors or steroid-related pneumatosis intestinalis were incidentally reported. The signal

Abbreviations: Pneumatosis intestinalis, (PI); immune-related adverse event, (irAE); immune checkpoint inhibitor, (ICI); FDA Adverse Event Reporting System, (FAERS); computed tomography (CT); programmed cell death 1 ligand 1, |(PD-L1); reporting odds ratio, (ROR); proportional reporting ratio, (PRR); information component, (IC); empirical Bayesian geometric mean, (EBGM); adverse drug reaction, (ADR); confidence interval, (CI); cytotoxic T lymphocyte antigen-4, (CTLA-4); programmed death receptor-1, (PD-1).

detected in five kinds of immune checkpoint inhibitors and six kinds of steroids implied a positive correlation between the drugs and adverse events.

Conclusion: Steroids might be the etiologic factors in the current case of pneumatosis intestinalis. Reports supporting the role of steroids in suspected cases of pneumatosis intestinalis can be found in literature databases and the FAERS database. Even so, as documented in FAERS, immune checkpoint inhibitors-induced pneumatosis intestinalis should not be excluded.

KEYWORDS

pneumatosis intestinalis, steroid, lung carcinoma, immune-related adverse events, FAERS, immune checkpoint inhibitor

1 Introduction

Pneumatosis intestinalis (PI) is an uncommon condition characterized by accumulating radiologically detected submucosal or subserosa gas cysts in the gastrointestinal wall (Heng et al., 1995). PI is associated with severe life-threatening complications. Clinical manifestations of PI range from asymptomatic to fatal, and its symptomatology includes abdominal pain, abdominal distention, nausea, vomiting, diarrhea, and constipation (Wang et al., 2018; Ling et al., 2019). Etiological factors of PI include intestinal diseases, systemic diseases, pulmonary diseases, medications, iatrogenic causes, and trauma (Lee and Wu, 2019).

Recently, a patient presented to our hospital with squamous lung carcinoma and PI secondary to prednisone use due to immune-related adverse events (irAEs). Accurate diagnosis of PI prevents unnecessary abdominal surgeries. In addition, immune checkpoint inhibitors (ICIs) increase patients' immunities. Consequently, clinicians are increasingly confronted with irAEs requiring steroid management. Therefore, we performed a literature search for published cases of PI associated with ICIs or steroids. In addition, we reviewed the FDA Adverse Event Reporting System (FAERS) database to identify additional cases of steroids-induced or ICIs-induced PI.

2 Case description

A 62-year-old male smoker with a 20-pack-year smoking history was admitted to the Department of Clinical Oncology at Peking University International Hospital complaining of hemoptysis in November 2020. Positron emission tomography/computed tomography (CT) revealed a 21 mm × 25 mm mass on the superior lobe of the right lung with enlarged mediastinal, bilateral hilar, and right supraclavicular lymph nodes. Further histopathological and molecular testing confirmed the diagnosis of squamous lung carcinoma in the absence of driver mutations. Immunohistochemical staining showed programmed cell death 1 ligand 1 (PD-L1) expression in 40% of the tumors. Standard platinum-based chemotherapy, including paclitaxel liposomes and carboplatin, was initiated. Contrast-enhanced CT scans revealed no responses after two cycles. According to the multidisciplinary team, the patient received two doses of 200 mg sintilimab in a three-week cycle with concurrent standard platinum-based chemotherapy. CT evaluation showed a partial response, with approximately 80% reduction in the size of the primary pulmonary lesions. The patient underwent definitive 60 Gy of thoracic radiotherapy with standard fractionation (2 Gy/fraction) between 4 March 2021, and 7 April 2021. Radiological evaluation revealed durable clinical responses. Subsequently, sintilimab treatment as consolidation was started 5 weeks after completing a 3-week course of radiotherapy.

After the third cycle of sinitilimab, the patient experienced dizziness, fatigue, nausea, and loss of appetite. Endocrinological examinations and brain Magnetic Resonance Imaging results suggested combined hypothyroidism and secondary adrenocortical insufficiency induced by sinitilimab. Hormone replacement therapy was administered, including physiological replacement doses of glucocorticoids and thyroxine. The patient's symptoms disappeared rapidly, and laboratory data spontaneously improved. Two months later, the fourth sintilimab infusion was administered. On 28 October 2021, total body CT showed continued partial response, with new consolidation. Based on multidisciplinary team, pneumonitis was diagnosed as a mild form of grade II (according to CTCAE 4.0). In addition to sintilimab discontinuation, systemic high-dose glucocorticoid therapy was prescribed (60 mg intravenous methylprednisolone daily for 7 days, followed by 40 mg oral methylprednisolone daily for 7 days, tapered gradually). Repeat CT showed improvement after 4 weeks, without tumor progression.

Unfortunately, the patient was admitted to our hospital due to progressive abdominal distension for 2 weeks on 17 December 2021. His vital signs were stable on arrival. However, a physical examination revealed hypoactive bowel sounds. Although the abdomen was non-tender with a tympanic percussion note. Abdominal X-Ray and CT examination suggested massive gas accumulation in the right half of the colon, gas in the intestinal wall, and free air under the diaphragm (Figures 1A, B). The patient was diagnosed with pneumoperitoneum and PI. Leukocytosis or C-reactive protein elevation was absent on blood film examination. However, renal function, liver function, and electrolytes, including potassium and sodium, were normal. Endocrinological examination revealed normal thyroid function, and serum cortisol and adreno cortico tropic hormone levels were within the lower limit. Physiological Hormone replacements with levothyroxine and prednisone acetate were administered daily to treat hypothyroidism and secondary adrenocortical insufficiency. Conservative management was recommended after a consultation between a gastrointestinal surgeon and a gastroenterologist. Parenteral nutrition, gastrointestinal decompression, and oxygen inhalation (3 mL/min) were initiated, and oral antibiotics

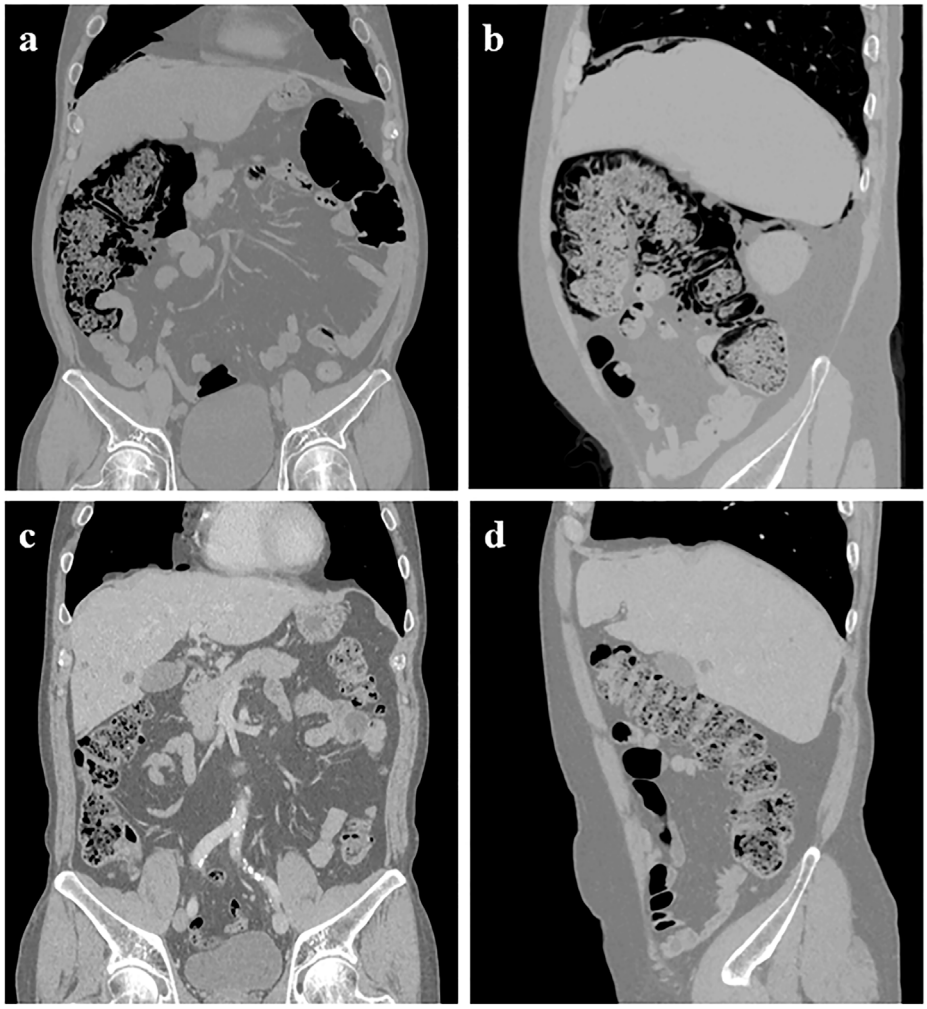

FIGURE 1
Abdominal CT showed pneumoperitoneum under the right diaphragm, extensive intramural air in the ascending colon, and hepatic flexure with massive air accumulation in the colon 6 weeks after starting high-dose methylprednisolone **(A–B)**. However, these findings had almost completely disappeared on abdominal CT taken at the outpatient clinic **(C–D)**.

(metronidazole) were used for therapeutic purposes. Flatulence and abdominal distention improved after treatment was initiated. CT showed reduced findings of gas in the abdomen, and the patient resumed a normal diet before discharge (Figures 1C, D). After sintilimab discontinuation, CT revealed a durable clinical response with residual actinic fibrosis. The patient has been in excellent condition without further anti-cancer therapies and immunotherapy for approximately a year.

3 Literature review of steroid-related PI cases

3.1 Methods

A literature review of the MEDLINE/PubMed and Web of Science Core Collection databases was conducted using the following retrieve terms: (Pneumatosis Intestinalis) AND "drug-induced" or "adverse event*" or "adverse reaction*" or "adverse drug reaction*" or "ADR." In addition, reports assessing ICIs and steroids as suspected drugs for PI were included.

3.2 Results

Six studies on steroid-related PI (Galm et al., 2001; Han et al., 2002; Patel et al., 2014; Ozturk et al., 2017; Lee and Wu, 2019; Nunomiya et al., 2021) were included. Patient characteristics, medication therapies, treatments, prognosis, and outcomes are summarized in Table 1. Eight cases of PI secondary to the combined therapy of steroids and other cytotoxic, immunosuppressive agents (Galm et al., 2001; Patel et al., 2014; Ozturk et al., 2017; Nunomiya et al., 2021) were identified, and two cases of PI secondary to steroid monotherapy (Han et al., 2002; Lee and Wu, 2019). In addition, six patients had underlying hematopoietic and lymphoid system conditions (Galm et al., 2001; Patel et al., 2014); one patient had lung adenocarcinoma, another had

TABLE 1 The information of patients with pneumatosis intestinalis.

No.	Sex/ Age (yr)	Underlying disease	Suspected drug	Dose	Steroid duration (day)	Abdominal symptoms	Radiologic findings	Infection	Location	Treatment	Re-challenge	Outcome
1(Nunomiya et al., 2021)	M/72	Lung adenocarcinoma	Bevacizumab, pemetrexed	Bevacizumab (15 mg/kg), pemetrexed (500 Mg/m^2)	N.A	Asymptomatic	Perforation; pneumatosis in the intestinal wall; no intraportal venous gas	None	Transverse colon	Observation; Supportive care	None	Resolved
2(Ozturk et al., 2017)	M/61	Nasopharyngeal cancer	Docetaxel, fluorouracil	Docetaxel (140 mg), fluorouracil (a total dosage of 7,000 mg)	N.A	Abdominal pain, bilious vomiting	Pneumatosis in the intestinal wall; gas in the portal vein, at the periphery of the liver parenchyma, and in the mesenteric veins	None	N.A.	Exploratory operation; Supportive care	N.A.	Resolved
3(Galm et al., 2001)	M/31	Acute T-lymphoblastic leukemia	Cyclophosphamide, mercaptopurine, cytosine arabinoside, prednisone	N.A.	N.A.	Abdominal pain	Free air under the diaphragm; pneumatosis in the intestinal wall	None	N.A.	Parenteral nutrition and antibiotics	Yes	Recurrence
4(Galm et al., 2001)	F/38	Chronic myelogenous leukemia	Daunorubicin, vincristine, dexamethasone	N.A.	N.A.	Asymptomatic	Free air under the diaphragm; pneumatosis in the intestinal wall; pneumoretroperitoneum; pneumomediastinum	None	Right colon	Parenteral nutrition and antibiotics	None	Resolved
5(Galm et al., 2001)	M/58	Lymphoma	Cyclophosphamide, vincristine, prednisone, dexamethasone, BCNU, etoposide, cytosine arabinoside, melphalan	N.A.	N.A.	Asymptomatic	Free air under the diaphragm; pneumatosis in the intestinal wall	None	The ascending and transverse colon	Parenteral nutrition and antibiotics	N.A.	Resolved
6(Galm et al., 2001)	F/64	Lymphoma	Cyclophosphamide, doxorubicin, vincristine, prednisone	N.A.	10	Diarrhea, abdominal pain and nausea	Pneumatosis in the intestinal wall	Yes	Distal jejunum, proximal ileum, and left hemi-colon	Parenteral nutrition and antibiotics	Yes	Cyclophosphamide was readministered because of the second relapse, no further episodes
7(Galm et al., 2001)	F/49	Aplastic anemia	Ciclosporin A, prednisone	N.A.	N.A.	Fever	Pneumatosis in the intestinal wall, pneumoretroperitoneum, and pneumomediastinum	Yes	N.A.	Parenteral nutrition; antibiotics	None	Died from respiratory failure
8(Patel et al., 2014)	M/14	Lymphoma	Cyclophosphamide, vincristine,	N.A.	5	Abdominal pain and distention		None	The ascending	Ileostomy	N.A.	Resolved

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TABLE 1 (*Continued*) The information of patients with pneumatosis intestinalis.

No.	Sex/Age (yr)	Underlying disease	Suspected drug	Dose	Steroid duration (day)	Abdominal symptoms	Radiologic findings	Infection	Location	Treatment	Re-challenge	Outcome
			daunorubicin, methotrexate, prednisone				Free air under the diaphragm; pneumatosis in the intestinal wall		and transverse colon			
9(Han et al., 2002)	M/38	Nephrotic syndrome	Prednisone	50 mg daily for 4 months 60 mg daily for 8 weeks and tapered to 20 mg daily	400+	Abdominal discomfort	Free gas in the retroperitoneal space and mediastinum; pneumatosis in the intestinal wall	None	Right colon	Conservative treatment; oxygen inhalation; metoclopramide	None	Resolved
10(Lee and Wu, 2019)	M/50	Superior mesenteric artery syndrome, hypersensitivity pneumonitis	Hydrocortisone, dexamethasone, methylprednisolone	Hydrocortisone (100 mg Q8H intravenous 5 days); Dexamethasone (4 mg TID oral 27 days); Methylprednisolone (40 mg BID intravenous 5 days)	37	Abdominal distention and vomiting	Pneumoperitoneum, pneumoretroperitoneum, and pneumatosis in the intestinal wall	None	N.A.	Peritoneal drainage and antibiotics	N.A.	Resolved

nasopharyngeal cancer (Ozturk et al., 2017; Nunomiya et al., 2021), and the remaining two had nephrotic syndromes (Han et al., 2002) and superior mesenteric artery syndromes (Lee and Wu, 2019). Symptoms were commonly identified, including abdominal pain and distention, diarrhea, nausea, and vomiting. Free air under the diaphragm, pneumatosis in the intestinal wall, and perforations and gas in other veins were the most reported radiologic findings. In the series report, parenteral nutrition and antibiotics were administered and two cases had associated infections (Galm et al., 2001). Two patients underwent exploratory (Ozturk et al., 2017) and ileostomy surgeries (Patel et al., 2014), while the remaining patients received supportive or conservative treatment. The conservative treatment included oxygen inhalation, metoclopramide, and peritoneal drainage (Han et al., 2002; Lee and Wu, 2019; Nunomiya et al., 2021). It showed that the majority of PI cases occurred within 1 month after suspected drug administration. Most cases were resolved; however, a patient died from respiratory failure (Galm et al., 2001).

4 FAERS analysis

4.1 Methods

Pneumatosis intestinalis was the preferred term in pharmacovigilance retrieval in the FAERS database, with a time range between the first quarter of 2005 and the third quarter of 2022. Four algorithms, including the reporting odds ratio (ROR), proportional reporting ratio (PRR), information component (IC), and empirical Bayesian geometric mean (EBGM), were used to calculate pharmacovigilance signals (van Puijenbroek et al., 2002). The "*a*," "*b*," "*c*" and "*d*" represented case numbers including the suspected drug and the adverse drug reactions (ADRs), case numbers including suspected ADRs with other drugs, case numbers including suspected drug with other ADRs, and case numbers including other drugs and other ADRs, respectively. The equations and criteria for the four algorithms were as follows: $ROR=(a/b)/(c/d)$, 95%CI = $e^{\ln(ROR)\pm1.96(1/a+1/b+1/c+1/d)0.5}$, (Criteria:95% CI > 1, $a\geq2$); $PRR=(a/(a+c))/(b/(b+d))$, $\chi^2=\Sigma((a-(a+b)(a+c)/(a+b+c+d))^2/((a+b)(a+c)/(a+b+c+d)))$ (Criteria: PRR≥2, $\chi^2\geq4$, $a\geq3$); IC = $\log_2 a(a+b+c+d)/((a+c)(a+b))$, IC025 = $e^{\ln(IC)-1.96(1/a+1/b+1/c+1/d)0.5}$ (Criteria: IC025 > 0); EBGM = $a(a+b+c+d)/((a+c)(a+b))$, EB05 = $e^{\ln(EBGM)-1.64(1/a+1/b+1/c+1/d)0.5}$ (Criteria: EB05 ≥ 2, $a>0$).

4.2 Results

A total of 1,272 cases of pneumatosis intestinalis related to ICIs ($n = 62$) or steroids ($n = 1,210$) were recorded in the FAERS database between 2005 and 2022. Therapeutic medication included five ICIs (ipilimumab, nivolumab, pembrolizumab, atezolizumab, and avelumab) and six steroids (dexamethasone, prednisone, betamethasone, hydrocortisone, methylprednisolone, and prednisolone). Demographic information, including reporting years and reporters, and patient information regarding age, sex, outcome, and onset time were listed in Table 2. The report numbers and algorithm signals for both groups are listed in Table 3. In ICIs group, the IC signal of the Ipilimumab, and the ROR, the IC, and the EBGM signals of the Nivolumab met the criteria. All four algorithms

TABLE 2 The demography and patient information of ICIs-related or steroids-related pneumatosis intestinalis in FAERS.

Category	Group	ICIs	Steroids	Total
Reporting Year (n = 1,272)	2005–2010	0	32	32 (2.52%)
	2011–2013	1	113	114 (8.96%)
	2014–2016	1	107	108 (8.49%)
	2017–2019	20	410	430 (33.81%)
	2020–2022	40	548	588 (46.23%)
Reportor (n = 1,272)	Consumer	7	120	127 (9.98%)
	Other health-professional	6	411	417 (32.78%)
	Pharmacist	6	5	11 (0.86%)
	Physician	40	342	382 (30.03%)
	Unknown	3	332	335 (26.34%)
Age (n = 1,272)	<18	0	494	494 (38.84%)
	18–44	1	76	77 (6.05%)
	45–64	12	220	232 (18.24%)
	65–74	26	60	86 (6.76%)
	75–84	15	52	67 (5.27%)
	>85	6	13	19 (1.49%)
	Unknown	2	295	297 (23.35%)
Sex (n = 1,272)	Female	17	389	406 (31.92%)
	Male	40	452	492 (38.68%)
	Unknown	5	369	374 (29.4%)
Onset time (n = 137)	0-10 days	7	38	45 (32.85)
	11-30 days	5	41	46 (33.58%)
	31-240 days	20	20	40 (29.20%)
	>240 days	2	4	6 (4.38%)
Outcome (n = 2,256)	congenital anomaly	0	11	11 (0.49%)
	Death	14	540	554 (24.56%)
	Disability	1	1	2 (0.09%)
	hospitalization - initial or prolonged	43	479	522 (23.14%)
	life-threatening	9	111	120 (5.32%)
	other serious (important medical event)	49	997	1,046 (46.37%)
	required intervention to prevent permanent impairment/damage	0	1	1 (0.04%)

showed positive signals for the rest drugs in each group. Besides, the FAERS analysis in our research only yielded 138 effective time to onset records and the median time was 92.72 days.

5 Discussion

Reports suggest that PI correlates with drug therapy (particularly prednisone therapy and α-glucosidase inhibitors), chemotherapy, molecular targeted therapy, and immunosuppressive agents (Hisamoto et al., 2006; Shinagare et al., 2012; O'Rafferty et al., 2014). However, the presence of non-specific symptoms increases the likelihood that PI is misdiagnosed or missed in the absence of imaging studies and that current morbidity estimates are inaccurate. Consequently, herein we present the case of a 63-year-old male patient with irAEs who developed PI after prednisone therapy. A literature review and a FAERS database exploration focusing on PI post steroids or ICIs were performed to identify a specific causative agent.

TABLE 3 Case numbers and detected signals of ICIs-related or steroids-related pneumatosis intestinalis.

Group	Drug	N (%)	ROR (95% CI)	PRR (χ^2)	IC (IC025)	EBGM (EB05)
ICIs (n = 62)	Ipilimumab	7 (0.55%)	1.71 (0.81)	3.59 (1.71)	2.06 (0.77)	0.37 (1.71)
	Nivolumab	21 (1.65%)	2.4 (1.56)	3.68 (2.4)	16.92 (1.25)	0.81 (2.38)
	Pembrolizumab	22 (1.73%)	4.14 (2.72)	6.31 (4.14)	51.88 (2.04)	1.34 (4.11)
	Atezolizumab	10 (0.79%)	3.99 (2.14)	7.42 (3.98)	22.24 (1.99)	1.07 (3.97)
	Avelumab	2 (0.16%)	6.3 (1.57)	25.23 (6.3)	8.91 (2.65)	0.66 (6.29)
Steroids (n = 1,210)	Prednisolone	460 (36.16%)	44.36 (39.99)	49.21 (44.16)	15107.04 (5.11)	4.61 (34.6)
	Betamethasone	3 (0.24%)	3.31 (1.07)	10.27 (3.31)	4.82 (1.72)	0.56 (3.3)
	Dexamethasone	151 (11.87%)	13.4 (11.35)	15.81 (13.38)	1,603.61 (3.64)	3.08 (12.48)
	Methylprednisolone	278 (21.86%)	62.52 (55.08)	70.96 (62.07)	14470.07 (5.75)	5.07 (53.9)
	Prednisone	213 (16.75%)	14.57 (12.64)	16.79 (14.55)	2411.41 (3.72)	3.23 (13.16)
	Hydrocortisone	105 (8.25%)	55.3 (45.41)	67.34 (54.92)	5278.47 (5.71)	4.69 (52.2)

N, case numbers; ROR, reporting odds ratio; CI, confidence interval; PRR, proportional reporting ratio; IC, information component; IC025, the lower limit of the 95% two-sided CI of the IC; EBGM, empirical Bayesian geometric mean; EB05, the lower 90% one-sided CI of EBGM.

The precise mechanisms leading to PI has yet to be elucidated (Shinagare et al., 2012). The consideration causes are now classified into the following categories. 1) Increased intra-abdominal pressure: factors including intestinal surgery, trauma, colonoscopy, obstruction, tumors, ischemic necrosis, and inflammatory reactions, may increase intraluminal pressure which potentially leads to mucosal dissection (Coriat et al., 2011). 2) Increased intra-pulmonary pressure: increased pressure and alveolar rupture could result into the introduction of air along vascular channels in the mediastinum, tracking downward to the aorta and portal system, and then to the intestinal wall (Azzaroli et al., 2011). 3) Microbial theory: bacteria could penetrate the intestinal wall through increasing the mucosa permeability, decompose nutrients, and produce gas, which leads the development of pneumatosis (Young et al., 1996; Honne et al., 2010). 4) Intestinal mucosal vascular injury: antiangiogenic drug and microangiopathy disrupt the intestinal wall by necrosis of the serosa (Coriat et al., 2011; Chang and Marzan, 2015; Nakagawa et al., 2015).

PI after steroid-containing treatment was observed in patients with acute T-lymphoblastic leukemia, chronic myelogenous leukemia, lymphoma, aplastic anemia, nephrotic syndrome, and superior mesenteric artery syndrome (Table 1). Since numerous clinical conditions are associated with PI, there may be many mechanisms for its development. However, a unified theory has yet to be established for its mechanism (Gazzaniga et al., 2022). A potential mechanism is the immunosuppression by steroids that results in the atrophy of Peyer's patches, inducing loss of intestinal mucosal integrity and leading to intestinal infection or gas migration (Bhamidipati et al., 2014).

Since the approval of ipilimumab for melanoma treatment in 2011, ICIs have changed the use of therapeutics in solid and hematological malignancies (Schmitt et al., 2022); approximately 50% of patients with malignancies are eligible for ICIs treatment (Haslam and Prasad, 2019). A substantial number of patients treated with ICIs will experience so-called irAEs. The incidence of irAEs in programmed death receptor-1 (PD-1) and PD-L1 inhibitors is approximately 15%, while in cytotoxic T lymphocyte antigen-4 (CTLA-4) antibody therapy is approximately 35%, and in the combination of CTLA-4 and PD-1 antibodies is approximately 55%. (Arnaud-Coffin et al., 2019). Although nuanced and targeted treatment of irAEs is desirable, for most moderate-to-severe irAEs, guidelines recommend the initial use of steroids (Haanen et al., 2018). Therefore, the potential risk of steriods-related PI warrants further study.

In general, there is a clear gender difference in hormone-related adverse reactions, and similar disproportion were found in our study. Both the FAERS database and case reports indicate that male appear to be more susceptible to drug-induced PI than female. Interestingly, gender difference was not limited to the steroids. Several studies have indicated that biological differences in sex hormones, body composition, and glucose metabolism may contribute to the disparity. Nevertheless, gender difference in drug-related PI requires further investigation.

Suspected sintilimab-induced PI should not be excluded, although studies reporting this finding have yet to be published. Sintilimab is a domestic PD-1 inhibitor in China that was approved for squamous and non-squamous non-small cell lung cancer by the National Medical Products Administration (Zhang et al., 2022). The gastrointestinal tract is commonly affected by ICIs (Rajha et al., 2020). However, normal bowel movement was observed in our patient during the six cycles of treatment with sintilimab. This suggests that PI likely correlated with methylprednisolone administration.

Oral steroid preparations tend to be highly bioequivalent (Francisco et al., 1984). The systemic bioavailability of prednisone and prednisolone are similar. Varying preparations of methylprednisolone also tend to be bioequivalent, although their oral and rectal absorption is uneven, in a relative bioavailability range from 50% to 90% (Garg et al., 1979). The pharmacokinetics of steroids in diseases and pathophysiological conditions, including severe liver disease, cystic fibrosis, end-stage kidney disease, hemodialysis, nephrotic syndrome, hyperthyroidism, obesity, and pregnancy, are diverse. In our case report, the patient had none of the above-mentioned conditions or off-label medication usage.

The diagnosis of PI mainly relies on imaging and endoscopy, which might easily lead to misdiagnosis and missed diagnosis because of the low incidence and non-specific clinical manifestations. Clinicians should pay attention to PI, collect medical history in detail, and analyze carefully. When imaging examination reveals free gas in the abdominal cavity but lacks symptoms of peritoneal irritation, PI should be considered as a possibility in order to diagnose and treat patients more rationally and avoid unnecessary surgical procedures.

Conservative treatments for PI, including administering oxygen at high concentrations, fasting, and antibiotics, are recommended for individuals with clinical manifestations of the condition (Feuerstein et al., 2014). CT scans are more sensitive to the accurate diagnosis of PI than plain radiographs, increasing the potential for identifying life-threatening conditions (Di Pietropaolo et al., 2020). PI without evidence of further intra-abdominal pathology does not necessitate laparotomy (Galm et al., 2001). PI complicated by bowel obstruction or ischemia tends to require emergency surgical intervention, which correlates with a higher clinical severity score (including degrees of pain, fever, tenderness, diarrhea, blood *per rectum*, and hypotension) (Yang et al., 2022). In our case, the absence of peritonitis, ischemia, and perforation, enabled conservative treatment with ceftriaxone, omeprazole, and sandostatin. Complete resolution of the PI was achieved following prednisone decrement and conservative therapy. However, this resolution should not preclude putting patients on the surgical alert list as the patients are still at risk for perforations and ischemia.

To our knowledge, this is the first study to evaluate irAE treatment-related PI. The study also compared the onset of PI secondary to different steroids in studies published and in the FAERS database. Therefore, this case report emphasizes the potential adverse events of PI associated with steroid use in the management of irAE. The onset of PI as an adverse event from steroids use requires further investigation.

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 patient provided their written informed consent to publish this case report.

Author contributions

TZ, MC, and LW conducted the case report, literature review, FAERS analysis, discussion, and prepared the manuscript. BZ and CP oversaw the FAERS data processing. LL, CT, ZZ, and JD supported the data analysis, review, and editing. JL supervised the study.

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Conflict of interest

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

Publisher's note

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Hematologic side effects of immune checkpoint inhibitor with or without chemotherapy in patients with advanced and metastatic gastrointestinal cancer: A systematic review and network meta-analysis of phase 3 trials

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Background: The regimens of immune checkpoint inhibitors (ICIs) alone or with chemotherapy are emerging as systemic therapy for patients with advanced and metastatic gastrointestinal cancers. However, the risk of treatment-related hematologic toxicity stays unclear.

Methods: We enrolled in phase 3 randomized clinical trials (RCTs) comparing PD-1, PD-L1, and CTLA-4 inhibitors in advanced and metastatic gastrointestinal cancers. The incidences of overall treatment-related adverse events (TRAEs), discontinuation, leukopenia, neutropenia, thrombocytopenia, and anemia were extracted for the Bayesian network meta-analysis. Analyses with poor convergence or low incidence were reported as incidences with 95% CIs instead.

Results: Sixteen phase 3 RCTs with 9732 patients who received systemic therapy were included. A total of 150 (1.54% [95% CI 1.31–1.80]) treatment-related death events were recorded, whereas 13 (0.13% [95% CI 0.08–0.22]) of them were hematologic. 0.24% (95% CI 0.12–0.48) patients received ICI plus chemotherapy were recorded for hematological deaths, 0.09% (95% CI 0.01–0.23) were for chemotherapy alone, and 0.05% were for ICI alone (95% CI 0.01–0.29). Febrile neutropenia was the most frequent cause of death in ICI with chemotherapy. For grade ≥3 TRAEs, we found nivolumab plus chemotherapy (OR 1.63 [95% CI 0.84–3.17]) had a higher risk than other treatments. Overall, ICI monotherapy led to fewer AEs than chemotherapy-based regimens in the analyses of leukopenia, neutropenia, thrombocytopenia, and anemia. Among the 11 treatments, toripalimab plus chemotherapy possessed the highest risk in any-grade leukopenia (OR 1.84 [95% CI 0.48, 6.82]) and neutropenia (OR 1.71 [95% CI 0.17, 17.40]) respectively. For grade ≥3 hematologic AEs, neutropenia (20.08% [95% CI 18.67–21.56]) related to ICI plus chemotherapy

was the most dominant. ICI plus chemotherapy was likely to increase the incidence than dosing these drugs alone.

Conclusion: Using ICI alone had a low incidence of causing hematologic mortality and AEs, while the combination with chemotherapy might magnify the side effects. Comprehensively, pembrolizumab plus chemotherapy and sintilimab plus chemotherapy were the safest regimens in terms of leukopenia and neutropenia respectively. This study will guide clinical practice for ICI-based chemotherapy.

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KEYWORDS

hematologic toxicity, immune checkpoint inhibitor, gastrointestinal cancer, phase 3 clinical trial, network meta-analysis

1 Introduction

Immune checkpoint inhibitors (ICIs) have emerged as an effective therapy for patients with advanced and metastatic gastrointestinal malignancies. Although the phase 3 KEYNOTE-062 study revealed no clinically meaningful benefit in first-line pembrolizumab plus chemotherapy vs. chemotherapy (Shitara et al., 2020), the use of ICIs still improves the survival outcomes in certain circumstances. For patients with higher programmed cell death ligand 1 (PD-L1) combined positive score (CPS) in esophageal and gastric cancer, the combination of ICI and chemotherapy was recommended as a higher category in National Comprehensive Cancer Network (NCCN) guidelines (Janjigian et al., 2021; Sun et al., 2021). Since KEYNOTE-177, pembrolizumab significantly longer progression-free survival (PFS) than chemotherapy as first-line therapy for microsatellite instability-high/mismatch repair-deficient (MSI-H/dMMR) metastatic colorectal cancer (Andre et al., 2020). The phase 2 CheckMate 142 study further demonstrated nivolumab plus low-dose ipilimumab had clinical benefit for MSI-H/dMMR colorectal cancer as well (Lenz et al., 2022). The preferred first-line treatment regimens are based on fluoropyrimidine and platinum which can induce severe hematologic side effects. The RAINFALL study reported the most common grade 3–4 adverse events (AEs) as neutropenia (27%) and anemia (14%) in patients who received fluoropyrimidine and cisplatin (Fuchs et al., 2019). Consistent with the results, Arai *et al* investigated the safety of fluoropyrimidine with platinum in advanced gastric cancer and found high rates in grade 3–4 leukocytopenia (17%), neutropenia (36%), and anemia (19%) as well (Arai et al., 2019). Meanwhile, blockade of programmed cell death 1 (PD-1) and PD-L1 could activate auto-reactive T-cells and auto-antibodies, then lead to a series of immune reactions (Matsumoto et al., 2020). Hematologic immune-related adverse events (irAEs) were less frequent but could be lethal (Tabchi et al., 2016). By constructing a large cohort of patients treated with ICI, previous research revealed the estimated incidence of hematologic irAE was 0.65% (Kramer et al., 2021). However, the risk of hematologic AEs from the combination of ICI and chemotherapy stays unclear.

Fast recognition and management of treatment-related adverse events (TRAEs) are crucial for patients with advanced cancer. Hematologic side effects are common in chemotherapy, thus understanding the potential risk of combining with ICI is necessary. To date, experienced oncologists have built an instructive framework of the solution to hematologic AEs related to chemotherapy (Crawford et al., 2004; Al-Samkari and Soff, 2021). As hematological side effects of ICIs are rare and difficult to diagnose, hematologic toxicities associated with ICI are poorly described (Schneider et al., 2021). Here, we enrolled the published phase 3 randomized clinical trials (RCTs) and conducted a Bayesian network meta-analysis. By analyzing neutropenia, leukopenia, anemia, thrombocytopenia, general AEs, and TRAEs (all-grade, grade ≥3), we provide a safety assessment of hematologic safety of PD-1, PD-L1, and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) inhibitors in monotherapy and combination with chemotherapy.

2 Methods

2.1 Literature search strategy and study selection

PubMed, Cochrane, and Embase databases were searched for relevant works. Several key search terms are listed as follows: 'immune checkpoint inhibitor', 'PD-1', 'PD-L1', 'chemotherapy', 'phase 3', and 'gastrointestinal cancer'. Papers published before 2 September 2022 were searched and screened for further analysis. The full search criteria are presented in Supplementary Table S2. We conducted this systematic review and network meta-analysis by following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Supplementary Table S1). The protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO CRD42022380150). Study screening was completed by two independent reviewers (JH and RX), and a third reviewer (ZZ) was consulted for any disagreement. The Inclusion criteria for trial selection were as below (1): Phase 3 RCTs enrolled advanced and/or metastatic gastric cancer, esophageal cancer, gastroesophageal junction cancer, and colorectal cancer (2); The intervention arms must include ICI (PD-1, PD-L1, or CTLA-4 inhibitor) and chemotherapy (3); Detailed data on hematologic and overall AEs were reported (4); Studies were published in English. Studies not meeting the inclusion criteria were excluded. Other exclusion

criteria were as below (1): Trials involved treatments other than ICI and chemotherapy (2); Studies exploring the efficacy and safety of sequential treatments (3); Literature such as case reports, cohort studies, conference abstracts, and letters were all excluded.

2.2 Data extraction and quality assessments

The following information was collected from each included study: study name, National Clinical Trial number, start year, study objective, treatment line, sample size, intervention regimens, overall TRAEs, treatment-related discontinuation, hematologic AEs (leukopenia, neutropenia, thrombocytopenia, and anemia), and death associated with hematologic AEs. AEs in any-grade and grade≥3 were defined as grade 1–5 and grade 3–5 respectively. TRAEs are defined as any AEs that confirmed by the investigators and might be caused by the study medication with reasonable possibility. All AEs are in accordance with the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (Freites-Martinez et al., 2021). We used the Cochrane Risk of Bias Tool to determine risk of the bias in each trial as high, unclear, or low (Higgins et al., 2011). Several score categories were noted: random sequence generation, allocation concealment, the blinding of participants and personnel, incomplete outcome data, the blinding of outcome assessment, selective reporting, and other biases (Supplementary Figure S1). Two authors (JH and RX) independently completed the process, and any disagreements in the assessment were resolved by a third investigator (QL).

2.3 Statistical analysis

To determine the appropriate model for network meta-analyses, we used a conservative approach to deal with between-study heterogeneity. If significant heterogeneity existed, we used the fixed effects model; otherwise, we used the Bayesian random-effects consistency model (Mills et al., 2013). Bayesian network modeling gives advantages to adapting to complex situations, by providing a straightforward method for probabilistic statements and treatment effect prediction (Salanti et al., 2011). The incidence of AEs was reported as an incidence with 95% confidence intervals (CIs), estimated through binomial probability. Odds ratios (ORs) with 95% CIs were used to analyze rate outcomes for data of AEs and discontinuation events. The inconsistency of evidence was shown in the inconsistency model comparisons (Lu and Ades, 2006). The surface under cumulative ranking curve (SUCRA) analysis was performed to calculate the AE ranking probability of each treatment regimen (Salanti et al., 2011). Between-study heterogeneity was estimated by the I^2 values of the consistency model if more than one comparison existed. I^2 values higher than 25%, 50%, or 75% suggested low, moderate, or high heterogeneity, respectively (Higgins et al., 2003).

To visualize the sample size and the number of comparisons, we used the "rjags" and "GeMtc" packages in R 4.0.3 (https://www.r-project.org/) and generated the Bayesian network modeling of AEs (Neupane et al., 2014). Incidences with 95% CI was calculated with the binconf () function in the "Hmisc" package. We also ran the analyses of heterogeneity and ranking probability in R. To identify the heterogeneity effects, the number of adaptations was set to 5000, and the sample iteration parameter was adjusted to 20,000.

3 Results

3.1 Eligible studies and baseline characteristics

The comprehensive search strategy identified 2202 records, and 357 records were eligible for further full-text screening (Figure 1). Following the selection criteria, 16 phase 3 RCTs with 9732 patients were included in the network meta-analysis (Bang et al., 2018; Shitara et al., 2018; Kato et al., 2019; Andre et al., 2020; Huang et al., 2020; Kojima et al., 2020; Shitara et al., 2020; Janjigian et al., 2021; Luo et al., 2021; Moehler et al., 2021; Sun et al., 2021; Chung et al., 2022; Doki et al., 2022; Kang et al., 2022; Lu et al., 2022; Wang et al., 2022). Among them, 3275 patients received ICI plus chemotherapy, 1926 patients received ICI alone, and 4531 received chemotherapy alone. Nine trials reported first-line therapy, six reported second-line therapy, and one reported third-line therapy. Most studies (15 of 16) investigated advanced and metastatic upper gastrointestinal tract cancer (esophageal cancer, gastroesophageal cancer, and gastric cancer), whereas only one study was associated with lower colorectal cancer. We identified ICIs as PD-1 inhibitors (pembrolizumab, nivolumab, camrelizumab, toripalimab, and sintilimab), PD-L1 inhibitors (avelumab), and CTLA-4 inhibitor (ipilimumab). The main characteristics of the included studies are presented in Table 1.

3.2 Overall incidence and cause of treatment-related deaths

To fully describe the landscape of treatment-related death events, we calculated the incidences of overall deaths and hematologic deaths. As shown in Table 2, a total of 150 (1.54% [95% CI 1.31–1.80]) treatment-related death events were recorded, whereas 13 (0.13% [95% CI 0.08–0.22]) of them were hematologic. Febrile neutropenia (0.06% [95% CI 0.03–0.13]) was the most frequent cause of death in ICI-based chemotherapy arms. By setting the population as a patient group who received allocated treatment, eight were correlated with ICI plus chemotherapy (0.24% [95% CI 0.12–0.48]), four were with to chemotherapy alone (0.09% [95% CI 0.01–0.23]), and only one was related to ICI alone (0.05% [95% CI 0.01–0.29]). The incidences of other hematologic TRAEs, including the decrease of white blood cells (WBCs), neutrophils, hemoglobin, and platelet were 0.01% (95% CI 0.01–0.06).

3.3 Network meta-analysis with the consistency and inconsistency model

Figure 2A illustrates the general network plots for 16 studies with hematologic safety assessment in 11 treatment regimens. The arms of chemotherapy alone and placebo plus

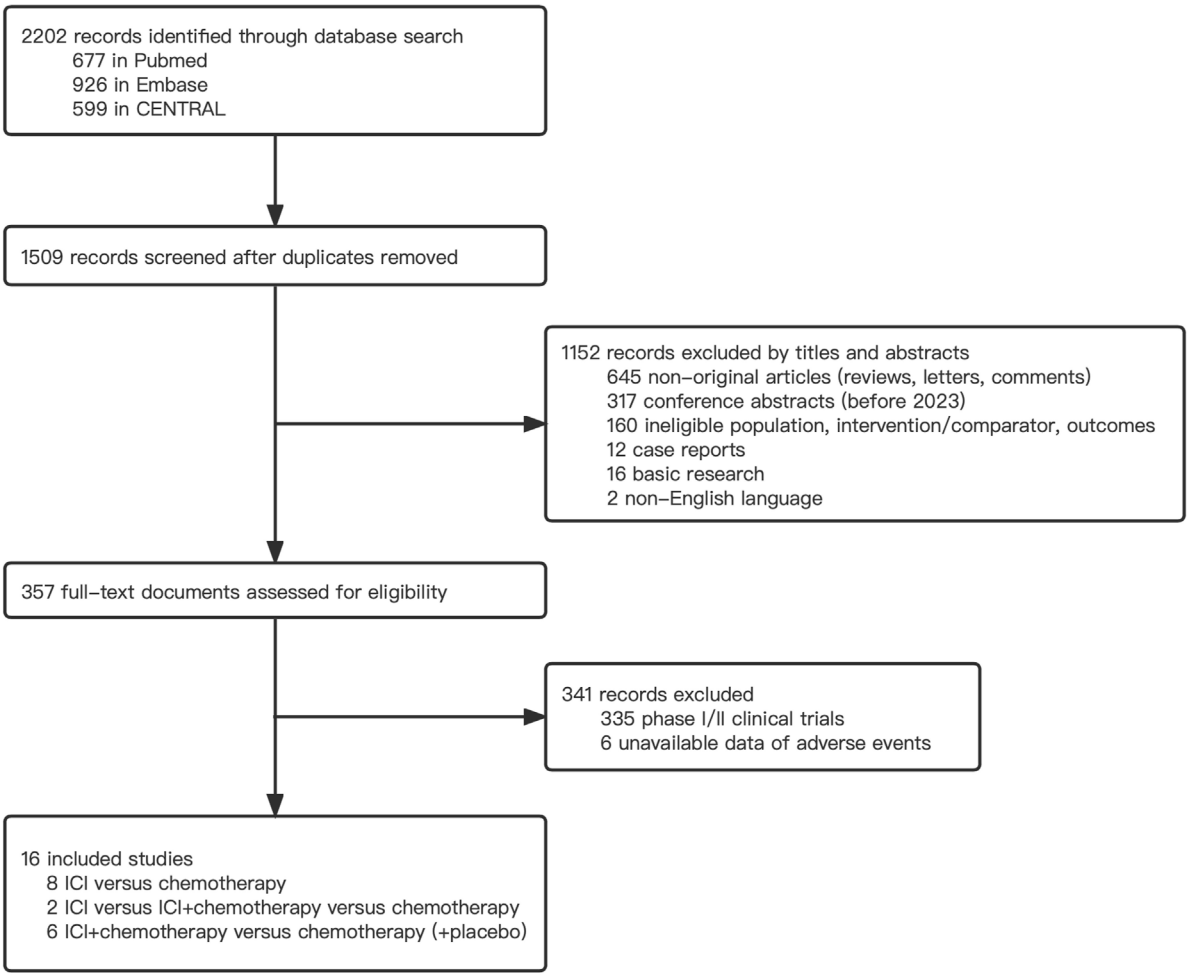

FIGURE 1
Flow chart of the study selection.

chemotherapy were stratified into a control arm for not receiving any ICI-based treatment interventions. As shown in Figure 2B, the OR of camrelizumab plus chemotherapy *versus* the control arm was 4.90 (95% CI 0.87–49.32) for TRAEs of any-grade, whereas the risk of sintilimab plus chemotherapy (OR 0.98 [95% CI 0.21–4.71]) and toripalimab plus chemotherapy (OR 1.01 [95% CI 0.07–12.84]) were consistent with chemotherapy. In terms of grade≥3 TRAEs, compared with the control arm, the combination of nivolumab (OR 1.63 [95% CI 0.84–3.17]) or pembrolizumab (OR 1.43 [95% CI 0.67–3.13]) with chemotherapy had an increased risk (Figure 2C). Using avelumab (OR 0.25 [95% CI 0.10–0.59]), camrelizumab (OR 0.37 [95% CI 0.11–1.17]), nivolumab (OR 0.12 [95% CI 0.04–0.39]), or pembrolizumab (OR 0.17 [95% CI 0.09–0.28]) alone deemed lower risk than chemotherapy. For AE-related discontinuation of treatment, pembrolizumab plus chemotherapy had the highest OR *versus* the control arm among the regimens (OR 1.91 [95% CI 0.68–5.32]), while sintilimab plus chemotherapy seemed to be the safest (OR 1.14 [95% CI 0.24–5.43]). The analyses of TRAEs of any grade, grade≥3 TRAEs, and discontinuation for AE were performed in inconsistency model to overcome the effect of heterogeneity.

We investigated the hematologic side effects by analyzing leukopenia, neutropenia, thrombocytopenia, and anemia of any grade (Figure 3). Overall, compared to chemotherapy, giving ICI alone or in dual had a significantly lower risk of arising hematologic AEs in these four terms. For leukopenia, toripalimab plus chemotherapy increased the risk most (OR 1.84 [95% CI 0.48–6.82]), while pembrolizumab (OR 1.00 [95% CI 0.37–2.40]) and sintilimab (OR 0.94 [95% CI 0.26–3.73]) plus chemotherapy harbored similar ORs when comparing to the control arm. The combination of toripalimab and chemotherapy also caused more neutropenia events (OR 1.71 [95% CI 0.17–17.40]). In contrast, the ORs of nivolumab (OR 1.09 [95% CI 0.29–4.15]) and sintilimab (OR 1.03 [95% CI 0.10–9.32]) plus chemotherapy *versus* the control arm were significantly lower. In terms of thrombocytopenia, all regimens of ICI and chemotherapy were deemed not significantly increased the risk of hematologic side effects. The ORs *versus* chemotherapy ranged from 0.80 (95% CI

TABLE 1 Studies evaluating safety of immune checkpoint inhibitors with or without chemotherapy.

	Study	Start year	Treatment line	Study objective	Treatment regimen (arm1/arm2/arm3)	No. of patients in safety assessment	Median age	ECOG PS 1
1	KEYNOTE-061 (NCT02370498)	2015	First-line	Advanced gastric or gastroesophageal junction cancer	Pembrolizumab/ chemotherapy	294/276	63/60	169/158
2	KEYNOTE-062 (NCT02494583)	2015	Second-line	Advanced gastric cancer	Pembrolizumab/ pembrolizumab plus chemotherapy/ chemotherapy	254/250/244	61/62/63	125/ 138/135
3	KEYNOTE-063 (NCT03019588)	2017	Second-line	Advanced gastric or gastroesophageal junction cancer	Pembrolizumab/ chemotherapy	47/44	61/61	33/35
4	KEYNOTE-177 (NCT02563002)	2016	Second-line	Advanced colorectal cancer	Pembrolizumab/ chemotherapy	153/143	63/63	78/70
5	KEYNOTE-181 (NCT02559687)	2015	First-line	Advanced esophageal cancer	Pembrolizumab/ chemotherapy	314/296	63/62	187/197
6	KEYNOTE-590 (NCT03189719)	2017	Second-line	Advanced esophageal cancer	Pembrolizumab plus chemotherapy/placebo plus chemotherapy	370/370	64/62	223/225
7	ATTRACTION-3 (NCT02569242)	2016	First-line	Advanced esophageal squamous cell carcinoma	Nivolumab/ chemotherapy	209/208	64/67	109/102
8	ATTRACTION-4 (NCT02746796)	2017	First-line	Advanced or recurrent gastric or gastroesophageal junction cancer	Nivolumab plus chemotherapy/placebo plus chemotherapy	359/358	64/65	167/168
9	CHECKMATE 648 (NCT03143153)	2017	First-line	Advanced esophageal squamous-cell carcinoma	Nivolumab plus chemotherapy/nivolumab plus ipilimumab/ chemotherapy	310/322/304	64/63/64	171/ 174/170
10	CHECKMATE 649 (NCT02872116)	2017	First-line	Advanced gastric, gastroesophageal junction, and esophageal adenocarcinoma	Nivolumab plus chemotherapy/ chemotherapy	782/767	62/61	462/452
11	ESCORT-1st (NCT03691090)	2018	Second-line	Advanced or metastatic esophageal squamous cell carcinoma	Camrelizumab plus chemotherapy/placebo plus chemotherapy	298/297	62/62	227/232
12	ESCORT (NCT03099382)	2017	First-line	Advanced or metastatic esophageal squamous cell carcinoma	Camrelizumab/ chemotherapy	228/220	60/60	182/176
13	JAVELIN Gastric 100 (NCT02625610)	2015	Third-line	Advanced or metastatic gastric or gastroesophageal junction cancer	Avelumab/chemotherapy	243/238	62/61	147/142
14	JAVELIN Gastric 300 (NCT02625623)	2015	First-line	Advanced gastric or gastroesophageal junction cancer	Avelumab/chemotherapy	184/177	59/61	119/124
15	JUPITER-06 (NCT03829969)	2019	First-line	Advanced esophageal squamous cell carcinoma	Toripalimab plus paclitaxel and cisplatin/ placebo plus chemotherapy	257/257	63/62	191/189
16	ORIENT-15 (NCT03748134)	2018	Second-line	Advanced or metastatic esophageal squamous cell carcinoma	Sintilimab plus chemotherapy/placebo plus chemotherapy	327/332	63/63	250/251

Abbreviations: ECOG, eastern cooperative oncology group; PS, performance status.

TABLE 2 Cause summary of death due to hematologic adverse events.

Drugs	Cause of TRAE death	Number	Study
Pembrolizumab	Decreased WBC count	1	KEYNOTE-181
Pembrolizumab plus chemotherapy	Febrile neutropenia	2	KEYNOTE-062
			KEYNOTE-590
Nivolumab plus chemotherapy	Febrile neutropenia	3	ATTRACTION-4
			CHECKMATE 649
Camrelizumab plus chemotherapy	Anemia	1	ESCORT-1st
Sintilimab plus chemotherapy	Myelosuppression	1	ORIENT-15
	Decrease in platelet count	1	
Chemotherapy with or without placebo	Decreased neutrophil count	1	KEYNOTE-181
	Febrile neutropenia	1	KEYNOTE-590
	Hemolytic anemia	1	ATTRACTION-4
	Decreased platelet count	1	ORIENT-15
Total		13	

Abbreviations: TRAE, treatment related adverse event; WBC, white blood cell.

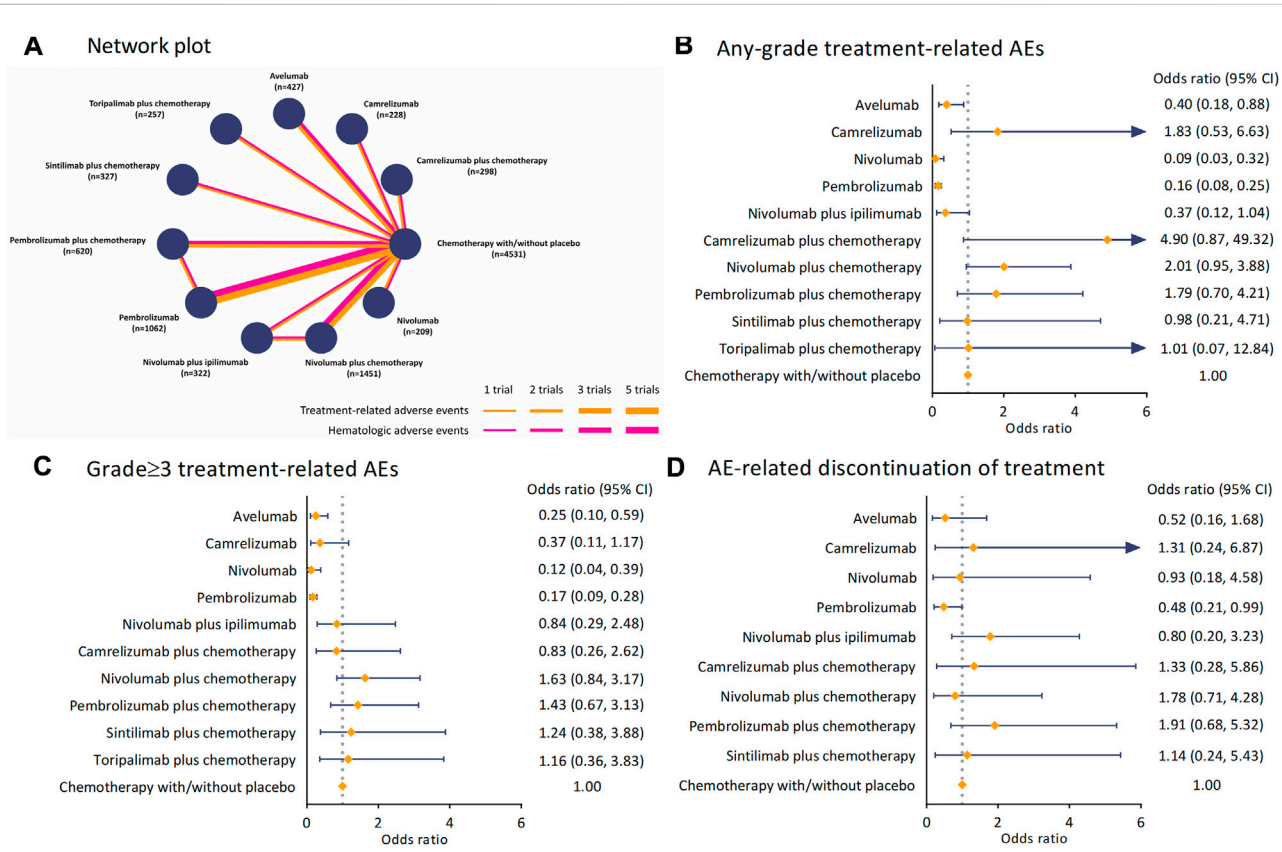

FIGURE 2
Network plot and pooled estimates of comparisons of TRAEs and AE-related discontinuation of treatment from ICIs. **(A)** Network plots for 16 studies with hematological safety assessment in 11 interventions. Each node refers to a treatment, and each line represents a type of head-to-head comparison. **(B)** ORs with 95% CIs for any-grade TRAEs. **(C)** ORs with 95%CIs for grade≥3 TRAEs. **(D)** ORs with 95%CIs for AE-related discontinuation of treatment. Abbreviation: TRAE, treatment-related adverse event; ICI, immune checkpoint inhibitor; OR, odds ratio; CI, confidence interval; AE, adverse event.

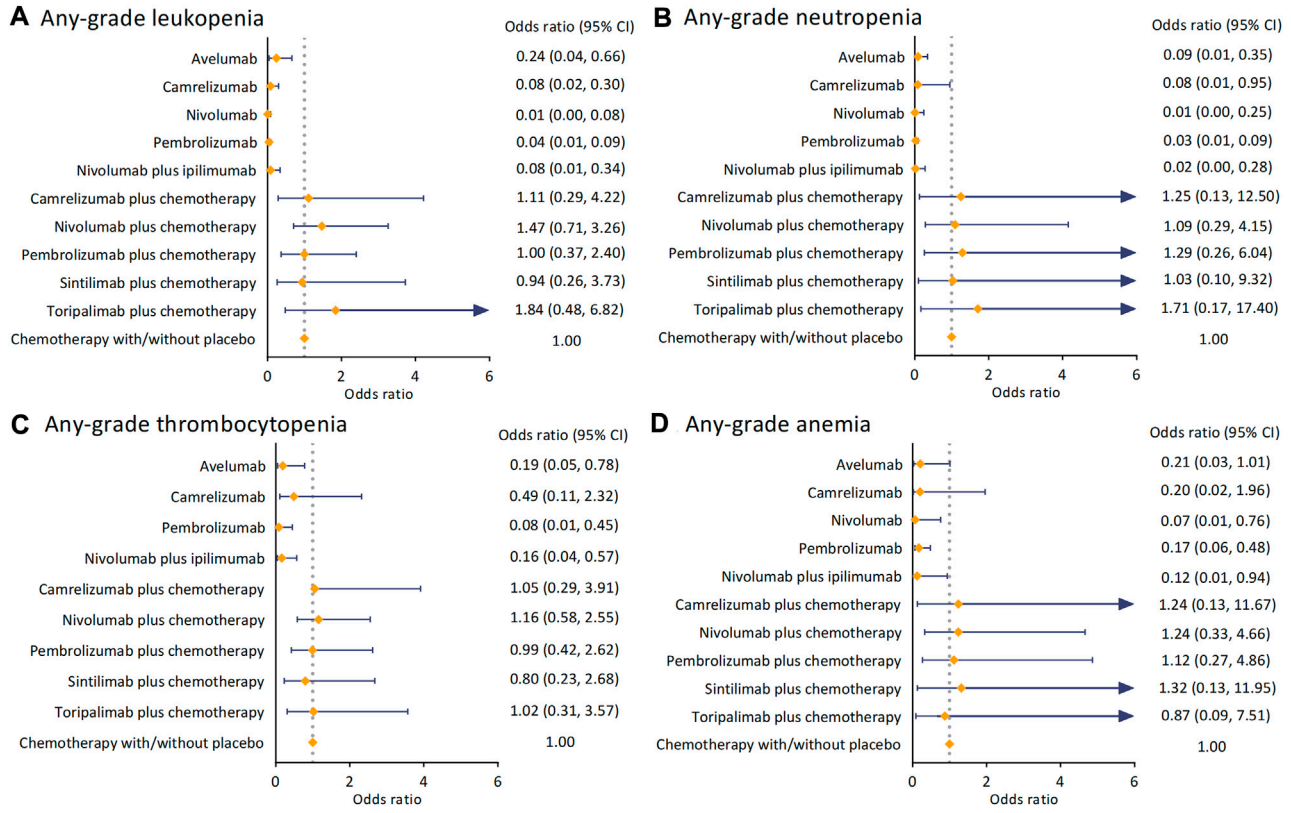

FIGURE 3
Pooled analysis of any-grade hematological AEs. **(A)** ORs with 95%CIs for any-grade leukopenia. **(B)** ORs with 95%CIs for any-grade neutropenia. **(C)** ORs with 95% CIs for any-grade thrombocytopenia. **(D)** ORs with 95% CIs for any-grade anemia. Abbreviation: AE, adverse event; OR, odds ratio; CI, confidence interval.

0.23–2.68) in sintilimab regimen to 1.16 (95% CI 0.58–2.55) in nivolumab regimen. We found camrelizumab with chemotherapy (OR 1.24 [95% CI 0.13–11.67]) and nivolumab with chemotherapy (OR 1.24 [95% CI 0.33–4.66]) had a consistent risk of causing anemia. Sintilimab plus chemotherapy increased this risk to OR 1.32 (95% CI 0.13–11.95).

3.4 Incidences of safety events in ICI with or without chemotherapy

To explore the potential additive safety risk of combination therapy, we stratified all the treatments (ICI, Chemotherapy +/- placebo, and ICI plus chemotherapy). The incidences of overall safety events and any-grade hematologic AEs were separately recorded and seen in Figure 4. The combination of ICI and chemotherapy caused more grade ≥3 TRAEs than using ICI or chemotherapy alone (Incidence 62.41% [95% CI 60.65–64.14]), as well as AE-related discontinuation events (Incidence 23.55% [95% CI 21.99–25.19]). By examining the emergence of leukopenia, neutropenia, thrombocytopenia, and anemia (Figure 4B), we found ICIs were associated with 2.02% (95% CI 1.44–2.82) - 6.54% (95% CI 5.52–7.73) any-grade AEs only. Giving chemotherapy caused 24.86% (95% CI 23.58–26.18) of patients with leukopenia and 27.08% (95% CI 25.81–28.39) with neutropenia, respectively. These incidences raised to 29.53% (27.91–31.20) for leukopenia and 33.97% (32.28–35.70) for neutropenia when dosing ICI with chemotherapy. Notably, giving ICI alone barely caused any grade≥3 leukopenia, neutropenia, and thrombocytopenia (Figure 4C). The most frequent grade≥3 hematologic AE for chemotherapy alone was neutropenia (Incidence 16.80% [95% CI 15.74–17.92]), and the combination therapy increased the incidence to 20.08% (95% CI 18.67–21.56). For grade ≥3 anemia, ICI with chemotherapy was accounted for 10.19% (95% CI 9.15–11.33) AEs. However, the incidences of grade≥3 leukopenia (Incidence 8.03% [95% CI 7.10–9.07]) and thrombocytopenia (Incidence 2.17% [95% CI 1.70–2.76]) in regimens of ICI and chemotherapy were almost consistent with chemotherapy alone.

3.5 Assessment of inconsistency, heterogeneity, and risk of bias

We calculated the variance deviation of random effects and inconsistency model and then presented the results in

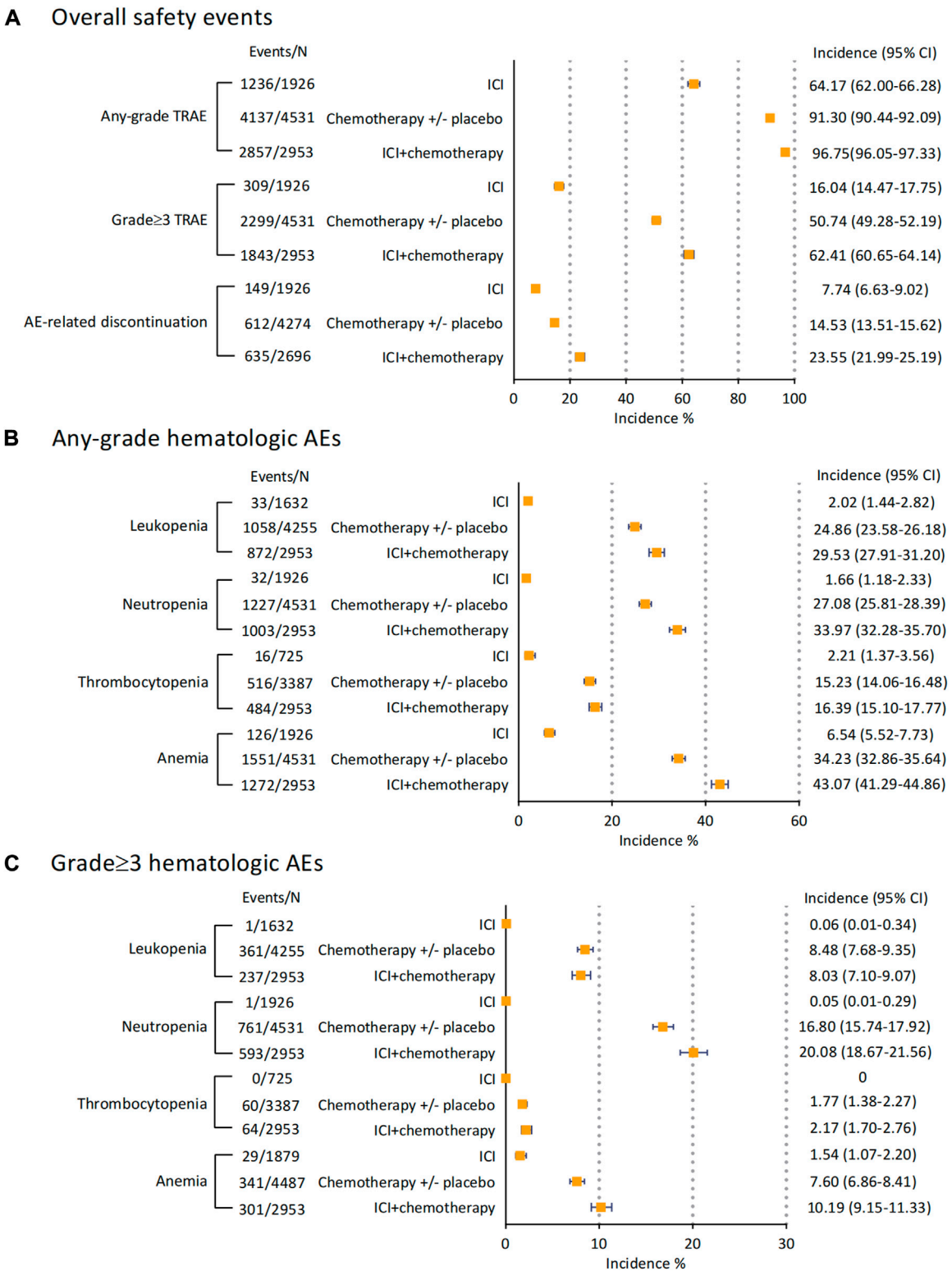

FIGURE 4
Incidences of overall safety events and hematological AEs of ICI monotherapy, ICI combined with chemotherapy, and chemotherapy with/without placebo. **(A)** incidences of overall safety events. **(B)** incidences of any-grade hematological AEs. **(C)** incidences of grade 3 hematological AEs. Abbreviation: AE, adverse event; ICI, immune checkpoint inhibitor.

Supplementary Table S3. The heterogeneity of general AEs and hematologic AEs was estimated and shown in Supplementary Table S4. The I^2 value suggested high heterogeneity in the analysis of anemia (I^2 = 83.6) and moderate heterogeneity in TRAEs of any grade (I^2 = 50.3), grade≥3 TRAEs (I^2 = 72.3), and discontinuation for AE (I^2 = 63.1). We assessed the quality assessment scored by the

Cochrane risk of bias tool in Supplementary Figure S1. Among the 16 studies, 11 of them had high risk of performance bias for poor blinding of participants.

4 Discussion

In this systematic review and network meta-analysis of ICI with or without chemotherapy, 16 trials for patients with advanced and metastatic gastrointestinal malignancies were evaluated. We assessed the categorized safety profile of PD-1, PD-L1, CTLA-4 inhibitors, and chemotherapy in ten ICI-based regimens. The general results indicate the principal findings:

(1) ICI caused much fewer general TRAEs and hematological TRAEs than ICI with chemotherapy or chemotherapy alone;
(2) The incidences of treatment-related hematological death were 0.24% in patients who received ICI with chemotherapy and 0.05% in patients received ICI alone;
(3) Febrile neutropenia was the most common cause of death in pembrolizumab plus chemotherapy and nivolumab plus chemotherapy;
(4) Toripalimab plus chemotherapy had the highest risk of leukopenia and neutropenia events, whereas sintilimab plus chemotherapy had the best safety in these two analyses;
(5) The incidence of hematologic AEs in ICI plus chemotherapy was higher than with the simple addition of ICI and chemotherapy.

Hematologic toxicities, commonly observed with chemotherapy, are the results of a cytotoxic effect on hematopoietic stem cells located in the bone marrow. Several cellular elements of the blood, including red blood cells (RBCs), WBCs, and platelets are involved. For decades, chemotherapy has been seen as a crucial regimen in patients with advanced and metastatic gastrointestinal cancer. To maximally ensure efficient dose and controllable tolerability, clinicians have greatly explored the hematologic side effects induced by chemotherapy and summarized a series of strategies (Ferreira et al., 2017; Castaman and Pieri, 2018). As ICIs are often given with chemotherapy (fluorouracil, capecitabine, oxaliplatin, cisplatin, *etc.*) in metastatic gastrointestinal cancer, understanding the potential hematological risk that combination therapy may arise can improve clinical practice.

Treatment-related death events are the most severe outcomes in the clinical experience. Among the 1926 patients who received ICI alone, only one death was recorded in pembrolizumab arm for decreased WBC count. Kramer *et al.* investigated hematological irAEs by enrolling 7626 patients treated with ICI, and only one had fatal outcomes (Kramer et al., 2021). Wang *et al.* explored the safety in 20,128 patients who received PD-1 and PD-L1 inhibitors, and the hematologic death rate was about 0.02% (Wang et al., 2019). Even though the hematological mortality of ICI is rare, clinicians should be aware of the potential side effects of the increased use of ICI. An observational study indicated both the low frequency of hematological toxicities (less than 1% in patients treated with anti-PD(L)-1) and the high rate of serious cases (grade ≥4 in 77% of patients) (Delanoy et al., 2019). Hematologic toxicities caused by ICI are divided into immune and non-immune. To date, no efficient technique has been reported to distinguish whether the hematological AEs are immune-related, which is crucial to the following treatment. Hematologic irAEs are highly life-threatening adverse reactions with a mortality rate reported to be 14% (Michot et al., 2019). The lethal causes of hematologic irAEs were identified as pancytopenia or aplastic anemia, autoimmune hemolytic anemia, hemophagocytic syndrome, and pure red cell aplasia.

The combination of ICI and chemotherapy may have additive hematologic side effects than using ICI or chemotherapy alone. Several meta-analyses explored the hematologic safety and tolerability of ICIs and chemotherapy respectively. Using PD-1/PD-L1 inhibitors alone, the rates of high-grade hematologic AEs were 0.2% for neutropenia, 0.5% for anemia, and 0.2% for thrombocytopenia (Nishijima et al., 2017). Using chemotherapy alone, the rates of high-grade hematologic AEs were 12.3% for neutropenia, 3.0% for anemia, and 3.4% for thrombocytopenia (Nishijima et al., 2017). Notably, when combined with chemotherapy, the rates of grade 3–5 hematologic AEs were higher than the summation of these two regimens (19.6% for neutropenia, 11.4% for anemia, and 6.8% for thrombocytopenia) (Zhou et al., 2021). Consistently, we found the combination of ICI and chemotherapy had a high incidence of leukopenia (29.53% [95% CI 27.91–31.20]), neutropenia (33.97% [95% CI (32.28–35.70]), and anemia (43.07% [95% CI 41.29–44.86]). Petrelli *et al.* enrolled 9324 patients with pan-cancer who received PD-1 and PD-L1 inhibitors, and indicated that severe neutropenia, thrombocytopenia, and febrile neutropenia were rare (Petrelli et al., 2018). ICIs were correlated with a moderate risk of anemia (10%) and a low risk of neutropenia and thrombocytopenia (0.9% and 2.8%), with negligible risk of febrile neutropenia (0.45%) (Petrelli et al., 2018). In the mortality analysis of this study, the hematological mortality of patients treated with chemotherapy alone was 0.09% (4/4531). However, the incidence of hematological death rose to 0.24% when combining ICI with chemotherapy. Unlike the non-specific cytotoxic effect of chemotherapy, PD-1/PD-L1 blockers have identical inhibitory effects on T-lymphocyte classes, B lymphocytes, NK cells, and macrophages. As a result, the putative mechanisms of ICI-associated hematological toxicities are described as autoantibody production, direct cytotoxicity, and excessive cytokine production (Kroll et al., 2022). The finds suggested that using ICI with chemotherapy needed careful estimation and caution for hematological safety. Here, by comprehensively analyzing, we present the hematological TRAEs that should be concerned when giving the regimes of each ICI plus chemotherapy.

- Pembrolizumab plus chemotherapy: neutropenia
- Nivolumab plus chemotherapy: leukopenia, anemia
- Camrelizumab plus chemotherapy: neutropenia, anemia
- Sintilimab plus chemotherapy: anemia
- Toripalimab plus chemotherapy: leukopenia, neutropenia

To date, no study has provided the hematologic safety profile of ICI with or without chemotherapy in advanced gastrointestinal cancer. Previous meta-analyses enrolled clinical trials of all-phase and focused on general safety (Yang et al., 2020; Guo et al., 2022). Our research included phase 3 trials only to avoid the risk of

reporting bias and quality control. By describing the incidence and network meta-analysis, we optimized the data presentation and ensured reporting accuracy. However, this work had several limitations that should be stated. First, we observed moderate to high heterogeneity in the analysis of anemia, TRAEs, and discontinuation for AE. The major contribution of heterogeneity was from the ATTRACTION-3 study. A possible reason for heterogeneity presence was the stratification of different chemotherapy regimens, which was designed to construct an entire and clear network. Second, this meta-analysis was performed at the study level instead of analyzing individual data. Third, to ensure drug tolerability, patients enrolled in these trials were screened before the recruitment. Therefore, in real-world experience, the patients may have more comorbidity than those who enrolled in clinical trials, potentially leading to a higher rate of side effects. Due to a very low incidence of hematologic irAEs and only numerical comparisons, the conclusion that ICI with chemotherapy could bring more mortaliteis may alter in future research. Finally, the results might be affected by the open-label design in 11 of 16 trials enrolled in this study, accounting for ascertainment bias.

5 Conclusion

In summary, using ICI alone had a low incidence of hematological AEs and mortality, however, with the combination of chemotherapy, the side effects could be magnified. Lethal febrile neutropenia was the most common cause for pembrolizumab and nivolumab with chemotherapy. Regimens of pembrolizumab plus chemotherapy and sintilimab plus chemotherapy were safe in arising leukopenia and neutropenia, respectively. These findings can optimize future trial designs and guide clinical pharmacology for investigations of ICI combination therapy.

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

JH, RX, and QX contributed to the study conception and design; JH, RX, ZZ, and QL performed the literature search, data extraction and analyzing; JH wrote the first draft of the manuscript; QX and YC revised the paper. All the authors have read and approved the final 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1163971/full#supplementary-material

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Immune cell death-related lncRNA signature as a predictive factor of clinical outcomes and immune checkpoints in gastric cancer

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Background: Immune cell death (ICD) is a type of tumor cell death that has recently been shown to activate and regulate tumor immunity. However, the role of ICD-related long non-coding RNAs (lncRNAs) in gastric cancer remains to be clarified.

Methods: We obtained 375 tumor samples from the Cancer Genome Atlas (TCGA) database and randomly assigned them to training and verification groups. LASSO and Cox regression analysis were utilized to identify ICD-related lncRNAs and establish a risk model. The changes in the immune microenvironment of the two groups were compared by examining the tumor-infiltrating immune cells.

Results: We established a tumor signature based on nine ICD-related lncRNAs. In light of the receiver operating characteristic and Kaplan–Meier curves, the prognostic values of this risk model were verified. Multivariate regression analysis showed that the risk score was an independent risk factor for the prognosis of patients in both the training cohort (HR 2.52; 95% CI: 1.65–3.87) and validation cohort (HR 2.70; 95% CI: 1.54–4.8). A nomogram was developed to predict the 1-, 3-, and 5-year survival of patients with gastric cancer, and the signature was linked to high levels of immunological checkpoint expression (B7-H3, VSIR).

Conclusions: An ICD-related lncRNA signature could predict the immune response and prognosis of patients with gastric cancer. This prognostic signature could be employed to independently monitor the efficacy of immunotherapy for gastric cancer patients.

KEYWORDS

gastric cancer, immune cell death, lncRNAs, prognostic signature, tumor immune microenvironment

Abbreviations: ANOVA, one-way analysis of variance; AUC, area under curve; GSEA, gene set enrichment analysis; LASSO, least absolute shrinkage and selection operator; lncRNAs, long non-coding RNAs

Background

Gastric cancer is the fifth most prevalent malignant cancer in the world, with the fourth highest mortality rate (Gao et al., 2022). Gastric cancers are usually at advanced stages when diagnosed, and the prognosis is poor (Sung et al., 2021). The main treatment methods for gastric cancer are surgery, radiotherapy, and chemotherapy (Fujiyoshi et al., 2021). In spite of the breakthroughs in immunotherapy in the past years (Wang et al., 2021a), the prognosis for gastric cancer patients remains poor. Thus, new biomarkers and preclinical models should be developed to identify more targets for the treatment and prognosis of gastric cancer.

Immune cell death (ICD) is a form of tumor cell death that has recently been revealed to activate and regulate tumor immunity. ICD promotes the recovery of a dysregulated antitumor immune system in the tumor microenvironment (TME). When ICD occurs, damage-associated molecular patterns (DAMPs) are released, which interact with dendritic cells (DCs) and promote their maturation. This improves the processing of engulfed cells and antigen presentation by DCs. DCs can stimulate certain T lymphocytes to exert cytotoxic effects on tumor cells *via* the mediation of antigen-presenting cells. ICD can eventually result in durable anticancer immunity in the host (Zhou et al., 2019). ICD-based anticancer drugs, such as belantamab, mafodotin (Tzogani et al., 2021), and lurbinectedin (Markham, 2020), are available for the treatment of melanoma and small cell lung cancer, respectively; however, ICD-related therapies for gastric cancer are rare. Further research is required to identify relevant biomarkers and conduct preclinical investigations.

Long non-coding RNAs (lncRNAs) are a type of RNA approximately 200 nucleotides in length. Due to their great specificity, they can be collected non-invasively, and their capacity to adapt to pathological and physiological changes in the human body makes them a research hotspot. LncRNAs are potential diagnostic and prognostic biomarkers for numerous diseases, and they can also influence the biological activity of cancer cells, including proliferation, migration, and invasion (Zhou et al., 2022). Recent research has demonstrated that lncRNAs can be utilized as reliable molecular markers for gastric cancer identification and progression prediction. LncRNAs may also aid in identifying new treatment and prognostic targets for gastric cancer (Fattahi et al., 2020).

We developed an ICD-associated lncRNA risk model that has good predictive value for the immune responsiveness and prognosis of patients with gastric cancer. This risk model could be utilized for the clinical management of patients with gastric cancer.

Materials and methods

Datasets and clinical information

For the purposes of this study, clinicopathological characteristics of gastric cancer and RNA sequence information were extracted from the Cancer Genome Atlas (TCGA, https://portal.gdc.cancer.gov/). Three patients were excluded due to incomplete clinical information. A total of 372 gastric tumor tissues and 32 normal tissues were obtained.

Identification of ICD-related lncRNAs

Based on previous research, 138 ICD-related genes were identified (Zhang and Chen, 2022). Pearson correlation analysis was performed to determine the association between lncRNAs and ICD-related genes. The criteria for Pearson correlation were a coefficient >0.4 and $p<0.001$.

Development and validation of a prognostic ICD-related lncRNA signature

Tumor samples were randomly divided into a training group and a verification group at a ratio of 1:2. The training cohort was used to build a risk model for ICD-related lncRNA, and the validation cohort was used to verify the risk model. First, univariate and multivariate Cox regression analyses were performed to identify prognosis-related lncRNAs. Then, ICD-related lncRNAs were screened using LASSO (least absolute shrinkage and selection operator)-based Cox regression. We calculated the risk score of the patients according to the following formula: risk score = expression of lncRNA1 b1lncRNA1 + expression of lncRNA2 b2lncRNA2 + expression of lncRNA bnlncRNAn. Based on the median risk score, the samples were assigned to a high-risk group or a low-risk group, and the effectiveness of the risk model was verified by receiver operating characteristic (ROC) and Kaplan–Meier (K-M) curves. Lastly, a nomogram was constructed to predict gastric cancer prognosis based on sample data.

The mRNA–lncRNA co-expression network

A co-expression network was constructed to show the relationship between ICD-related genes and ICD-related lncRNAs, and a Sankey diagram was created to depict the relationship among lncRNAs, mRNAs, and risk types.

GSEA and functional enrichment analysis

The 'limma' package was used to identify differentially expressed genes between normal and tumor tissues with cutoff criteria of <0.05 for the false discovery rate and >1 for |log2foldchange|. The gene expression data were interpreted by gene set enrichment analysis (GSEA) (http://www.broadinstitute.org/gsea) (Subramanian et al., 2005), and CIBERSORT (Barbie et al., 2009) was used to illustrate the tumor-infiltrating immune cells in gastric cancer.

Statistical analysis

Data analysis was performed using R, version 3.30. The *t*-test and Wilcoxon test were conducted for group comparisons, and one-way analysis of variance (ANOVA) was performed to compare the two samples. The model's prognostic value was evaluated by univariate and multivariate regression analyses, with $p< 0.05$ being considered statistically significant.

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TABLE 1 Characteristics of gastric cancer patients in the training cohort and validation cohort.

Characteristic	Training	Validation	*p*	Statistic
n	248	124		
Status, n (%)			0.792	0.07
Alive	153 (61.7%)	74 (59.7%)		
Dead	95 (38.3%)	50 (40.3%)		
Gender, n (%)			0.379	0.77
Female	93 (37.5%)	40 (32.3%)		
Male	155 (62.5%)	84 (67.7%)		
Race, n (%)			0.896	
Asian	50 (23.4%)	23 (21.1%)		
Black or African–American	7 (3.3%)	4 (3.7%)		
Native Hawaiian or other Pacific islander	1 (0.5%)	0 (0%)		
White	156 (72.9%)	82 (75.2%)		
Neoplasm histologic grade, n (%)			0.342	
G1	5 (2.1%)	5 (4.1%)		
G2	86 (35.5%)	48 (39.7%)		
G3	151 (62.4%)	68 (56.2%)		
Stage event pathologic stage, n (%)			0.766	1.14
Stage I	32 (13.7%)	18 (15.7%)		
Stage II	73 (31.2%)	38 (33%)		
Stage III	105 (44.9%)	45 (39.1%)		
Stage IV	24 (10.3%)	14 (12.2%)		
Stage pathologic T, n (%)			0.695	1.45
T1	13 (5.3%)	5 (4.1%)		
T2	48 (19.8%)	30 (24.8%)		
T3	113 (46.5%)	55 (45.5%)		
T4	69 (28.4%)	31 (25.6%)		
Stage pathologic N, n (%)			0.365	3.18
N0	71 (30.2%)	37 (31.1%)		
N1	62 (26.4%)	35 (29.4%)		
N2	56 (23.8%)	19 (16%)		
N3	46 (19.6%)	28 (23.5%)		
Stage pathologic M, n (%)			1.000	0
M0	221 (92.9%)	107 (93%)		
M1	17 (7.1%)	8 (7%)		
Time, median (IQR)	413.5 (209, 745)	477 (279, 795.75)	0.315	14393.5
Risk score, median (IQR)	0.18 (−0.08, 0.52)	0.18 (−0.1, 0.5)	0.982	15354
Age, median (IQR)	67 (58, 73)	68 (57, 74)	0.929	15215.5

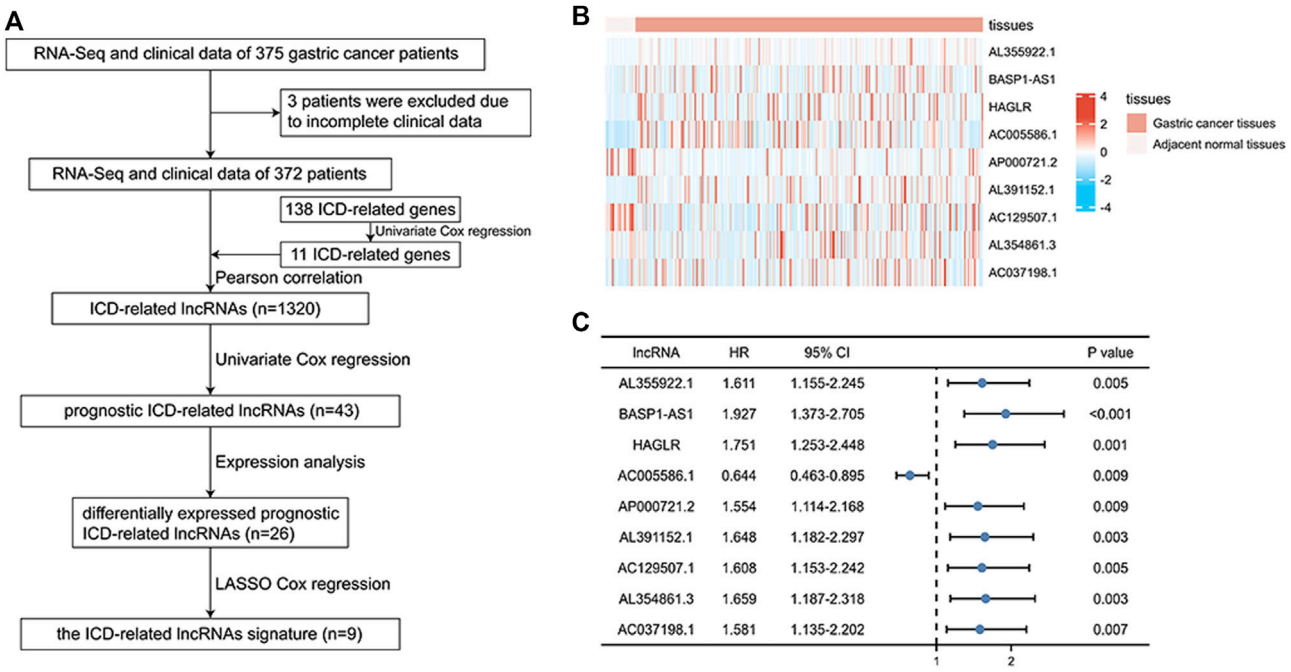

FIGURE 1
Identification of ICD-related prognostic lncRNAs between normal and gastric cancer tissue. **(A)** Flowchart for construction of the ICD-related lncRNA signature. **(B)** Heatmap of nine prognostic ICD-related lncRNAs in gastric cancer tissues and adjacent normal tissues. **(C)** Univariate Cox regression of nine prognostic ICD-related lncRNAs. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Results

The identification of ICD-related prognostic lncRNAs in normal and gastric cancer tissues

First, we collected data on gastric cancer from the TCGA database and obtained RNA sequences and clinicopathological information from 375 tumor tissues and 32 normal tissues. This sample set was randomly divided into a training cohort and a verification cohort in a ratio of 1:2. The characteristics of the samples in the two cohorts are shown in Table 1. It can be seen that there are almost no significant differences between the two cohorts. There were 138 ICD-related genes acquired from previous studies (Zhang and Chen, 2022; Supplementary Table S1). Eleven prognostic ICD-related genes were identified by univariate Cox regression analysis, and we obtained 1,320 ICD-related lncRNAs using Pearson correlation analysis. After repeated univariate Cox regression analyses, it was found that 43 ICD-related lncRNAs were associated with gastric cancer prognosis. The expression analysis results showed that 26 prognostic ICD-related lncRNAs were differentially expressed in the two datasets. Lastly, nine ICD-related lncRNAs were used to construct the signature by LASSO Cox regression. The operational flow chart is shown in Figure 1A, and the expression levels of the nine ICD-related lncRNAs in gastric cancer and adjacent normal tissues are shown in Figure 1B. The univariate Cox regression analysis results are shown in Figure 1C. In Figure 2A, the mRNA–lncRNA co-expression network illustrates the relationship between mRNA and lncRNA. For example, AC129507.1 is related to three mRNAs: CAV1, ELANE, and MAP1LC3C. The Sankey diagram in Figure 2B illustrates the relationship among ICD-related mRNA, lncRNA, and risk types. We found that AC005586.1 and AP00072102 exerted inhibitory effects against gastric tumors.

The establishment and verification of the ICD-related lncRNA signature

The signature was constructed using LASSO regression analysis. The risk score was computed using the following formula: (0.04217 × AL355922.1) + (0.13753 × BASP1-AS1) + (0.04922 × HAGLR) + (−0.08037 × AC005586.1) + (0.18179 × AL391152.1) + (0.48802 × AC129507.1) + (0.61776 × AL354861.3) + (0.0542 × AC037198.1) + (−0.37274 × AP000721.2). We used the median risk score to classify the cohorts into high and low risk groups, as shown in Figure 3A-B. After survival analysis, it was found that the survival probability of the high-risk group was larger than that of the low-risk group, and the difference was statistically significant (training cohort: HR = 2.52 (1.65–3.87), $p < 0.001$; verification cohort: HR = 2.70 (1.51–4.85), $p < 0.001$) (Figure 3C-D). In the training cohort, the AUC reached 0.685 at 1 year, 0.690 at 3 years, and 0.791 at 5 years; in the verification cohort, the AUC reached 0.703 at 1 year, 0.682 at 3 years, and 0.736 at 5 years (Figure 3E-F). These results confirm that the signature has good predictive effectiveness. To further verify the validity of the model, multivariate and univariate regression analyses of the training cohort and the verification cohort were conducted (Tables 2 & 3), and the results show that risk score is an important prognostic factor of gastric cancer.

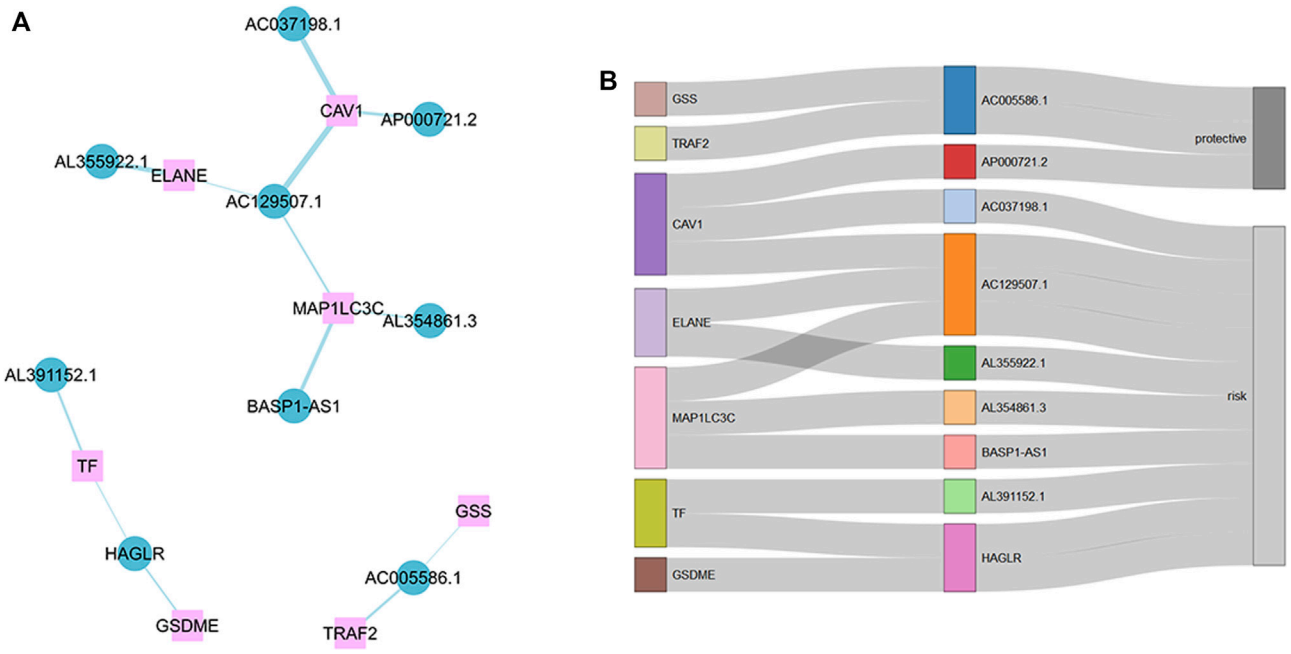

FIGURE 2
Messenger RNA (mRNA)–lncRNA co-expression network. **(A)** mRNA–lncRNA co-expression network of the ICD-related genes and selected ICD-related lncRNAs. **(B)** Sankey diagram showing connection degree between ICD-related lncRNAs and ICD-related genes.

Construction of a new nomogram with clinicopathological information

Univariate regression analysis of patients with gastric cancer demonstrates that old age, advanced tumor stage (stages III and IV), and high risk scores have a significant adverse effect on prognosis (Figure 4A). More detailed analysis is required to determine the efficacy of the signature. Accordingly, a nomogram was constructed to further verify the prognostic effect of the signature, based on the regression analysis results. The nomogram includes age, tumor stage, and risk score and can predict the 1-, 3-, and 5-year survival probability of gastric cancer patients with a C-index of 0.703 (0.679–0.727) (Figure 4B).

Differences in immune microenvironment between high- and low-risk groups

The difference in the molecular mechanisms between high- and low-risk groups can be determined by GSEA. In Figure 5, nine immune-related signaling pathways are associated with the signature, including the reactome interaction between L1 and ankyrins, Kyoto Encyclopedia of Genes and Genomes (KEGG) antigen processing and presentation, Biocarta MHC pathway, Biocarta TCRA pathway, WB inflammatory response pathway, Biocarta CTLA4 pathway, Biocarta Th1Th2 pathway, Biocarta IL5 pathway, and reactome PD-1 signaling. These results may provide a theoretical basis for future immunotherapy of gastric cancer.

We also studied the TME of patients with gastric cancer because it is closely connected with ICD (1, 13–17). The values obtained from all the procedures were combined, the CIBERSORT algorithms were applied, and the percentage of specific immune cells was estimated, as shown in Figure 6A. The heat map of immune cell expression in the high- and low-risk groups is shown in Figure 6B. The results of correlation analysis of various immune cells are displayed in Figure 6C-E, depicting the differential expression of immune cells that have invaded tumors in patients with gastric cancer. The expression level of naive B cells, monocytes, resting myeloid dendritic cells, activated mast cells, and eosinophils was higher in patients than that in the low-risk group. The expression level of M0 and M1 macrophages, resting NK cells, follicular helper T cells, and activated $CD4^+$ memory T cells was higher in patients than in the high-risk group. In addition, we examined the expression patterns of diverse immunological checkpoints in the high-risk and low-risk groups, and found that the difference between PD-L1 and VSIR was statistically significant (Figure 6F).

Discussion

ICD is a special type of cell death that has been found to connect tumor cells with the host's immune system. ICD can activate the immune system by releasing DAMPs, exerting potent anti-tumor effects, and potentially inducing long-lasting anti-tumor immunity in patients (Feng et al., 2022). ICD is a cell death mode with promising therapeutic prospects for gastric cancer (Liao et al., 2022), and identifying an ICD-related signature could pave the way for more effective treatments of gastric cancer. In our study, we obtained complete RNA-seq and clinical data of 372 patients from the TCGA. By reviewing the existing literature and applying univariate analysis, we identified 138 ICD-related genes, and of

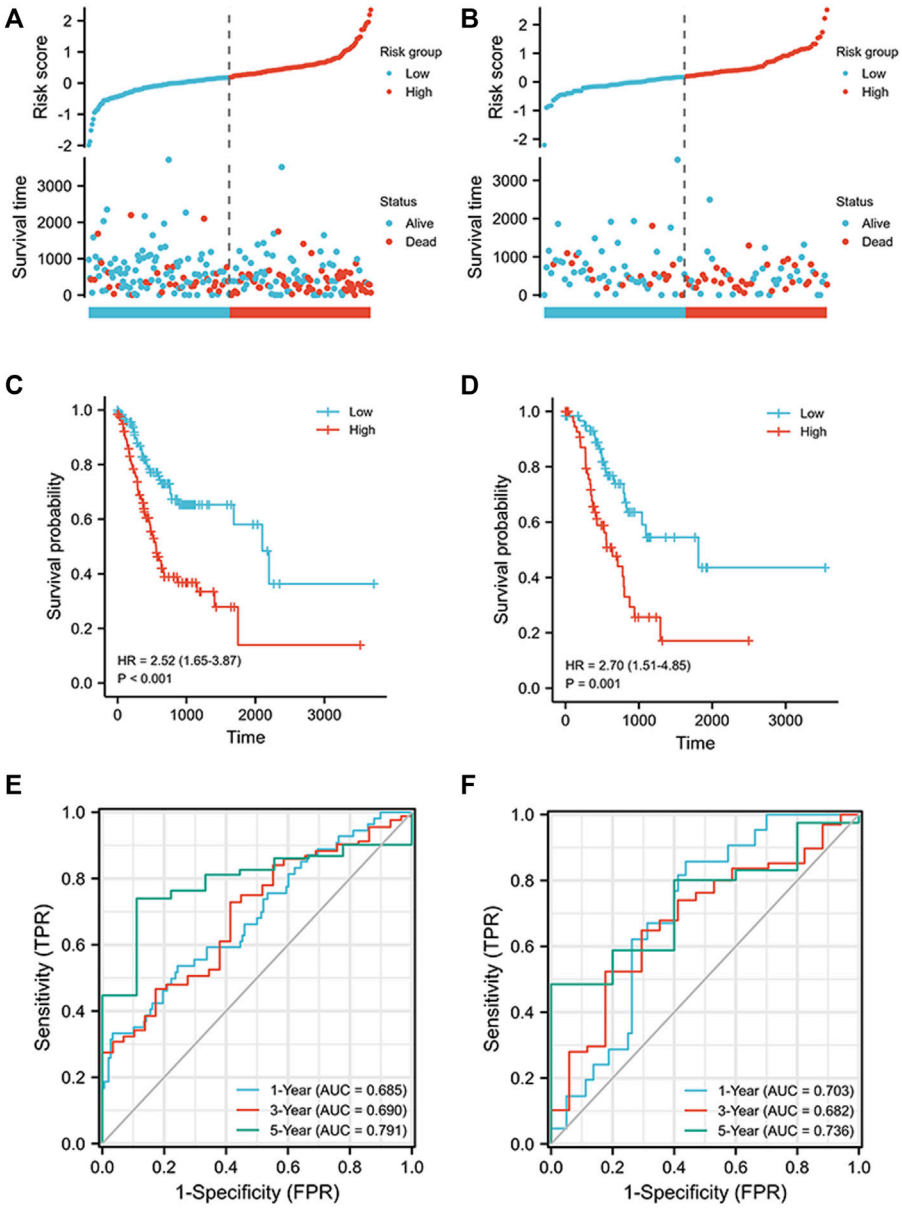

FIGURE 3
Prognostic analysis of ICD-related lncRNA signature in training and validation cohorts. **(A)** Distribution of risk scores in the training cohort and overall survival status, overall survival, and risk score. **(B)** Distribution of risk scores in the validation cohort and overall survival status, overall survival, and risk score. **(C)** Kaplan–Meier curves for the overall survival of patients in the high- and low-risk groups in the training cohort. **(D)** Kaplan–Meier curves for the overall survival of patients in the high- and low-risk groups in the validation cohort. **(E)** AUC of time-dependent ROC curves verifying the prognostic accuracy of risk scores in the training cohort. **(F)** AUC of time-dependent ROC curves verifying the prognostic accuracy of risk scores in the validation cohort.

these, 11 were prognosis-related. Next, 43 prognosis-related lncRNAs were obtained through Pearson correlation analysis and univariate COX regression analysis, and nine prognosis-related ICD-related lncRNAs were obtained through differential expression analysis and LASSO regression analysis to construct the signature. Further validation was performed through differential expression analysis in gastric cancer tissues and adjacent normal tissues. We found that the high-risk groups stratified by the signature had a better prognosis than the low-risk groups, and that the signature had good predictive value for prognosis. Lastly, we plotted K–M curves to predict the 1-, 3-, and 5-year survival of patients based on their risk score combined with their clinical characteristics. This signature helps doctors make better predictions of prognosis, which should help patients with gastric cancer get appropriate treatment.

A growing number of studies in recent years have shown that lncRNAs play an important role in the genesis and progression of many malignancies, as well as in the TME. In our signature, we identified nine lncRNAs, namely, AL355922.1, BASP1-AS1, HAGLR, AC005586.1, AP000721.2, AL391152.1, AC129507.1,

TABLE 2 Univariate and multivariate analyses of risk factors in the training cohort.

Characteristics	Total (N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	*p*-value	Hazard ratio (95% CI)	*p*-value
Risk Score	248	2.812 (2.023–3.908)	**<0.001**	2.688 (1.850–3.904)	**<0.001**
Age	246	1.028 (1.007–1.050)	**0.008**	1.031 (1.009–1.054)	**0.006**
Gender	248				
Male	155	Reference			
Female	93	0.674 (0.431–1.054)	0.084		
Race	214				
White	156	Reference			
Other	8	1.387 (0.553–3.484)	0.486		
Asian	50	0.718 (0.392–1.313)	0.282		
Neoplasm histologic grade	242				
G1-2	91	Reference			
G3	151	1.549 (0.995–2.410)	0.053		
Stage pathologic stage	234				
Stage I–II	105	Reference			
Stage III–IV	129	1.663 (1.074–2.574)	**0.022**	1.571 (1.011–2.441)	**0.045**

TABLE 3 Univariate and multivariate analyses of risk factors in the validation cohort.

Characteristic	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	*p*-value	Hazard ratio (95% CI)	*p*-value
Risk score	124	1.792 (1.157–2.776)	**0.009**	1.759 (1.123–2.756)	**0.014**
Age	123	1.016 (0.988–1.046)	0.260		
Gender	124				
Male	84	Reference			
Female	40	0.983 (0.550–1.758)	0.953		
Race	109				
White	82	Reference			
Asian	23	0.595 (0.233–1.520)	0.278		
Other	4	1.921 (0.589–6.265)	0.279		
Neoplasm histologic grade	121				
G1-2	53	Reference			
G3	68	1.055 (0.598–1.861)	0.854		
Stage event pathologic stage	115				
Stage I–II	56	Reference			
Stage III–IV	59	2.416 (1.288–4.533)	**0.006**	2.303 (1.225–4.331)	**0.010**

AL354861.3, and AC037198.1. We found that BASP1-AS1 was critical for the development and prognosis of melanoma (Li et al., 2021). Additionally, BASP1-AS1 has been shown to significantly affect the proliferation of glioma cells and may be a new target for glioma treatment (Xu et al., 2021). Many studies have indicated that HAGLR exerts an unfavorable effect on the prognosis

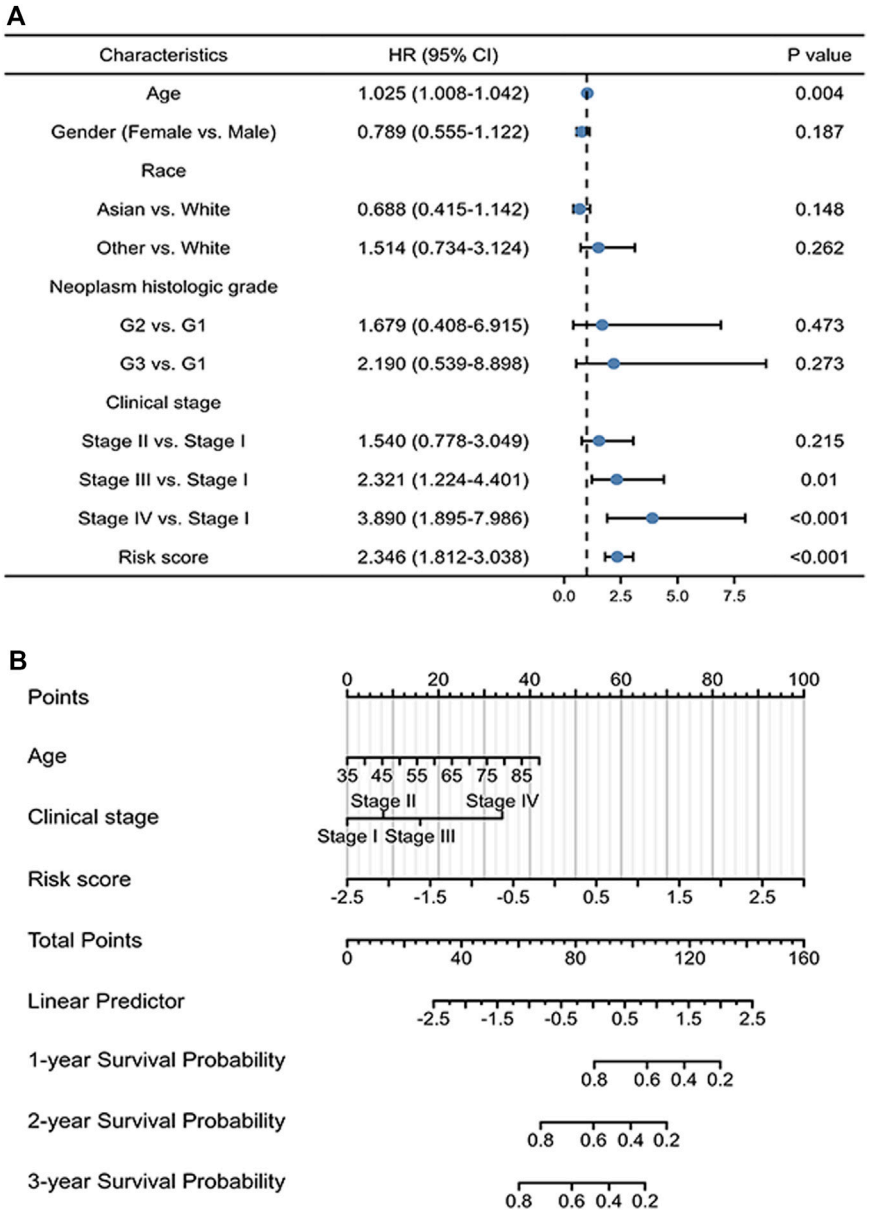

FIGURE 4
Prognostic value of the ICD-related lncRNA signature. **(A)** Multivariate Cox regression of patient characteristics and the signature as a whole. **(B)** Nomogram of the risk model with clinical information.

and treatment of gastrointestinal tumors. It enhances the resistance of gastric cancer to 5-fluorouracil by targeting the glycolysis pathway, which necessitates the development of new drugs (Hu et al., 2022). Furthermore, HAGLR promotes the occurrence and development of liver cell cancer (HCC) through miR-6785-5p, resulting in a poor prognosis (Li et al., 2020). Similarly, high HAGLR expression in colon cancer accelerates its progression (Sun et al., 2020). LncRNA AL391152.1 has been studied in gastric cancer, and results indicated that AL391152.1 was a novel glycolysis-related lncRNA that could accurately predict the overall survival time of gastric cancer patients (Zeng et al., 2022a). It has also been demonstrated that AL391152.1 is related to cellular aging and can effectively predict the response to immunotherapy in gastric cancer patients (Zeng et al., 2022b). In addition, another study used AL391152.1 to construct a gastric cancer risk score model (Liu et al., 2020). Together, these studies demonstrate that lncRNA AL391152.1 has broad prospects for application. Many studies have found that AC129507.1 plays a role in the development, immunotherapy, and prognosis of gastric cancer, which is consistent with our findings in the present research. Platelet activation-related lncRNA AC129507.1 can serve as a biomarker for prognosis and for the response of gastric cancer patients to immunotherapy (Yuan et al., 2022). Similar findings have been reported in other studies (Han et al., 2021; Zeng et al., 2022b). Three hypoxia-related lncRNA AC037198.1-associated molecular subtypes characterized by different prognoses and immune

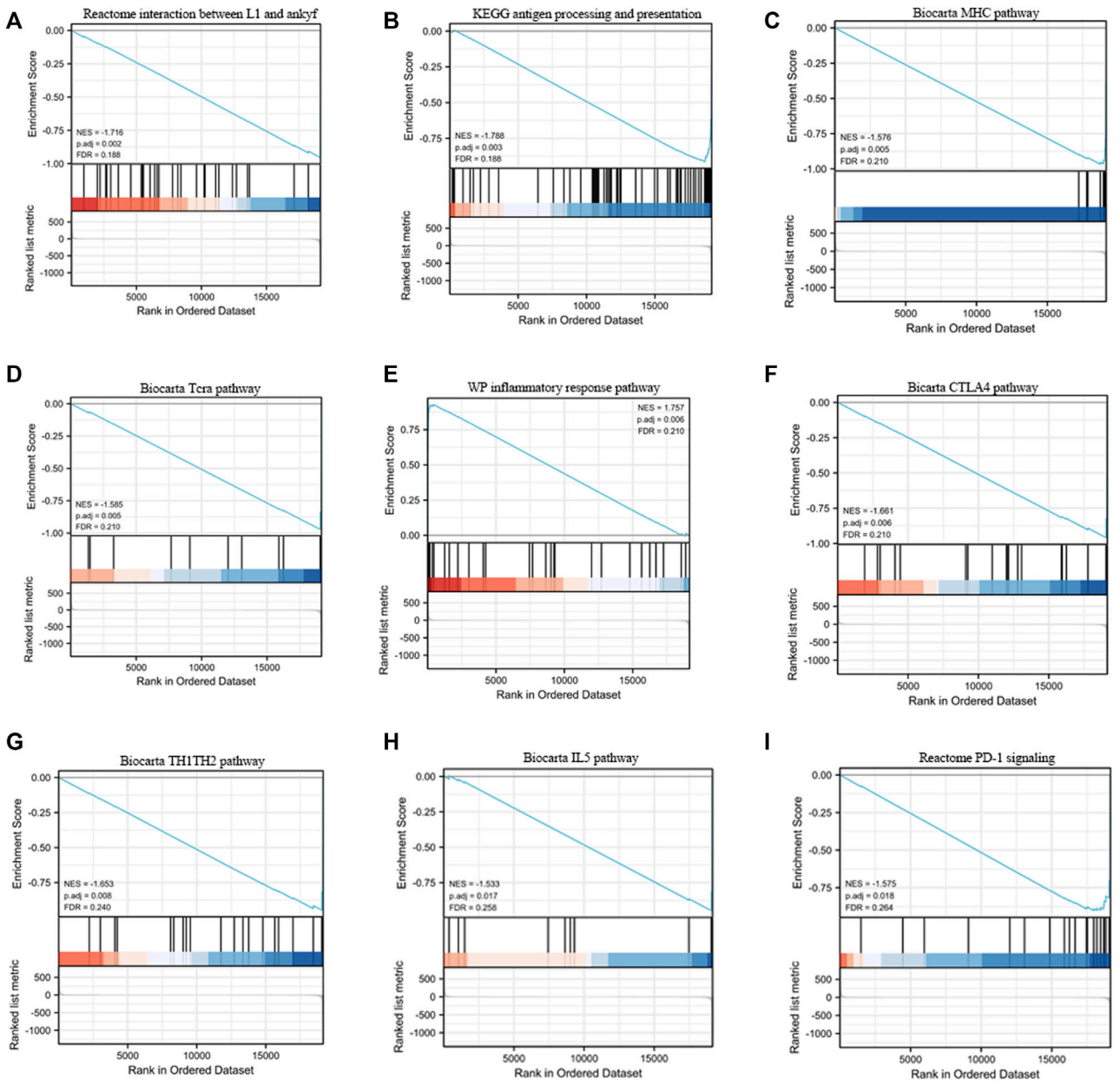

FIGURE 5
Gene set enrichment analysis (GSEA) of the ICD-related lncRNA prognostic signature. **(A)** Reactome interaction between L1 and ankyrins. **(B)** KEGG antigens and presentation. **(C)** Biocarta MHC pathway. **(D)** Biocarta TCRA pathway. **(E)** WB inflammatory response pathway. **(F)** Biocarta CTLA4 pathway. **(G)** Biocarta Th1Th2 pathway. **(H)** Biocarta IL5 pathway. **(I)** Reactome PD-1 signaling.

conditions have been identified, which can provide a theoretical basis for the improvement of clinical diagnosis and treatment of gastric cancer (Fan et al., 2022).

ICD-related lncRNAs have only been studied in liver cancer and stomach adenocarcinoma. He et al. screened 20 lncRNAs based on 33 ICD-related genes to construct a prognostic model. The model was helpful in classifying subtypes of liver cancer based on ICD-related lncRNAs and molecules, and for predicting the prognosis of patients with liver cancer and their therapeutic response to immunotherapy (He et al., 2022). Ding et al. screened five lncRNAs based on 34 ICD-related genes to construct a prognostic model for gastric adenocarcinoma. The model could predict the cumulative survival rate and guide individual treatment (Ding et al., 2022). The aforementioned studies all indicated that ICD was closely related to lncRNAs and to disease prognosis. In the present research, we constructed a different prognostic model based on the 138 ICD-related genes identified in the latest studies, and consistent results were obtained.

The immune system of cancer patients constantly fights cancer cells. As a result, some cancer cells have developed the ability to evade recognition and elimination by the immune system, which is referred to as “immune evasion” (Wang et al., 2022). Gastric cancer cells can

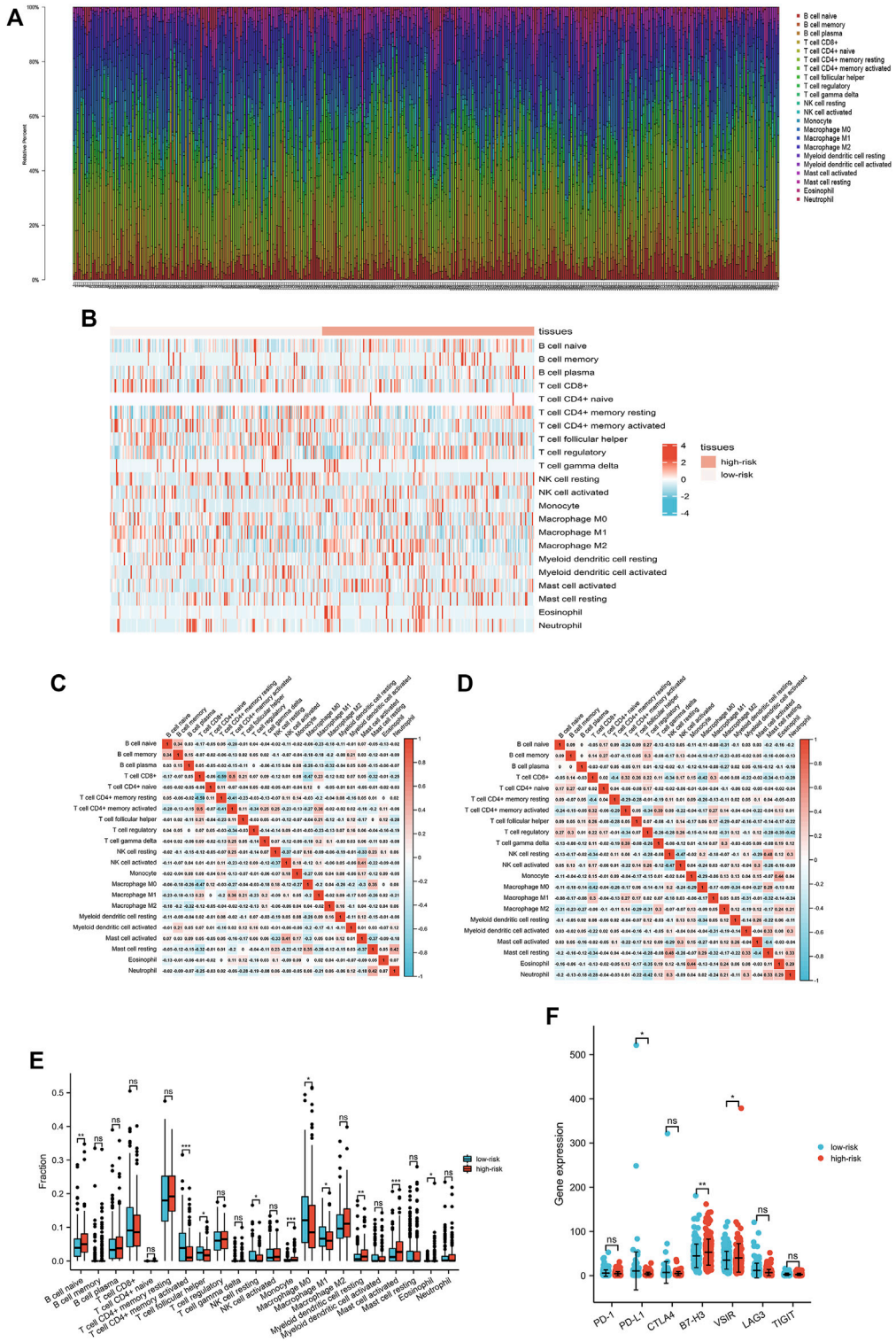

FIGURE 6
Interactions between the ICD-related lncRNA signature and immune regulation in patients with gastric cancer. **(A)** Degree of immune cell infiltration **(B)** Heatmap of tumor-infiltrating cells in low- and high-risk patients. **(C, D)** Correlation matrix of immune cells in gastric cancer. **(E)** Comparisons of immune cells between low- and high-risk groups. **(F)** Comparison of multiple immune checkpoints between low-risk and high-risk groups, including PD-1, PD-L1, CTLA4, B7-H3, VSIR, LAG3, and TIGIT.

avoid monitoring and attack by the immune system (Schreiber et al., 2011). Immune evasion is closely associated with the immune microenvironment, which could become a new aspect of research on gastric cancer treatment (Wang et al., 2021b; Ding et al., 2022; Gao et al., 2022; Han et al., 2022; Liao et al., 2022; Xu et al., 2022). Therefore, we analyzed the effect of ICD-related gene mutations on immune cell

infiltration in the high- and low-risk groups and found that there were statistically significant differences between the two groups in many immune cells. This finding demonstrates the effectiveness of the constructed risk model for guiding future immunotherapy regimens and the development of immunoreactive drugs. In addition, various signaling pathways, which may play a crucial role in the immune evasion of gastric cancer cells, could significantly affect the prognosis and drug resistance of gastric cancer patients. Therefore, we also performed a GSEA analysis on the high- and low-risk groups, and the results provide a new perspective for future signal pathway-related gastric cancer treatment.

Because the immune system plays a critical role in tumor development, scholars are seeking to extend the survival of patients with gastric cancer through innovative immunotherapy (Zhang et al., 2021). One of the most recent therapeutic strategies combines immunotherapy with immuno-checkpoint inhibitor and ICD-related therapies. Significant progress has been made in immuno-checkpoint inhibitors, and several drugs based on immune checkpoints have been developed in recent years (Zou et al., 2019). We found that certain immunological checkpoints (B7-H3 and VSIR) were overexpressed in high-risk patients. However, many individuals responded poorly to this therapeutic method, and some showed resistance. ICD-related therapy may bring new opportunities for the treatment of refractory gastric cancer.

Our research has some limitations. First, it is a bioinformatics analysis based on public databases. Although we downloaded samples from multiple databases, the data are still limited. Second, this study is a retrospective study, and the findings still need to be verified by further multicenter prospective cohort studies with large sample sizes.

Conclusion

We constructed a signature of nine ICD-related lncRNAs based on ICD-related genes and demonstrated its effectiveness in predicting the prognosis of patients with gastric cancer. The signature is strongly associated with the immune microenvironment, and our findings support a new vision and direction for immunotherapy in the treatment of gastric cancer.

Data availability statement

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

Ethics statement

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

Author contributions

YY and ZZ: conception and design. DS, AT, KZ, and XF: data curation. DS, ZZ, and YY: writing and revision of the manuscript. All authors contributed to the manuscript and approved the submitted version.

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Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1162995/full#supplementary-material

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Value assessment of PD-1/PD-L1 inhibitors in the treatment of oesophageal and gastrointestinal cancers

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Background: Evidence of efficacy and safety of programmed cell death 1 (PD-1) and programmed death ligand-1 (PD-L1) checkpoint inhibitors in oesophageal cancer (EC), gastric cancer (GC) and colorectal cancer (CRC) was inconsistent, obscuring their clinical application and decision-making. The aim of this study was to comprehensively evaluate the value of PD-1/PD-L1 inhibitors in EC, GC and CRC to select valuable PD-1/PD-L1 inhibitors, and to assess the association between the value and cost of PD-1/PD-L1 inhibitors.

Methods: A comprehensive search of trials of PD-1/PD-L1 inhibitors in EC, GC and CRC was performed in Chinese and English medical databases with a cut-off date of 1 July 2022. Two authors independently applied the ASCO-VF and ESMO-MCBS to assess the value of PD-1/PD-L1 inhibitors. A receiver operating characteristic (ROC) curve was generated to establish the predictive value of the ASCO-VF score to meet the threshold of the ESMO-MCBS grade. Spearman's correlation was used to calculate the relationship between the cost and value of drugs.

Results: Twenty-three randomized controlled trials were identified: ten (43.48%) in EC, five (21.74%) in CRC, and eight (34.78%) in GC or gastroesophageal junction cancer (GEJC). For advanced diseases, ASCO-VF scores ranged from −12.5 to 69, with a mean score of 26.5 (95% *CI* 18.4–34.6). Six (42.9%) therapeutic regimens met the ESMO-MCBS benefit threshold grade. The area under the ROC curve was 1.0 (p = 0.002). ASCO-VF scores and incremental monthly cost were negatively correlated (Spearman's ρ = −0.465, p = 0.034). ESMO-MCBS grades and incremental monthly cost were negatively correlated (Spearman's ρ = −0.211, p = 0.489).

Conclusion: PD-1/PD-L1 inhibitors did not meet valuable threshold in GC/GEJC. Pembrolizumab met valuable threshold in advanced microsatellite instability–high CRC. The value of camrelizumab and toripalimab may be more worth paying in EC.

KEYWORDS

PD-1/PD-L1 inhibitors, ESMO-MCBS, ASCO-VF, value, cost

Introduction

According to GLOBOCAN data, colon cancer, gastric cancer (GC), rectal cancer and oesophageal cancer (EC) are among the top 10 cancers in terms of incidence, and digestive system cancers have become one of the most serious disease burdens (Sung et al., 2021). In recent years, the use of programmed cell death 1 (PD-1) and programmed death ligand-1 (PD-L1) inhibitors in the treatment of digestive system cancers has been proven to improve the survival of patients and has become an important research topicality (Kang et al., 2017; André et al., 2020; Doki et al., 2022). However, our previous study found that the efficacy and safety of PD-1/PD-L1 inhibitors in EC, GC and colorectal cancer (CRC) were inconsistent (Ou et al., 2022), which extremely confused their clinical application and usefulness in aiding decision-making.

The goal of cancer treatment has changed from the traditional disease-centred strategy to a patient-centred strategy, and we should pay more attention to the comprehensive value (safety, quality of life, affordability, etc.) of the therapeutic regimen in addition to its efficacy. The value of anti-tumor drug is an integrated concept, including safety and efficacy, together with attributes such as quality of life, cancer-related symptoms and cost. It is a quantifiable concrete value that can reflect the personalized characteristics of the drug to meet the different preferences of patients. The skyrocketing price of new anti-tumour drugs (especially targeted therapy and immunotherapy drugs), combined with the high burden of cancer, has resulted in an urgent need to assess their value *versus* their cost. The American Society of Clinical Oncology (ASCO) and the European Society for Medical Oncology (ESMO) have developed and updated their conceptual frameworks to assess the benefit of new cancer therapies: the ASCO Value Framework (ASCO-VF) and the ESMO-Magnitude of Clinical Benefit Scale (ESMO-MCBS) (Cherny et al., 2015; Schnipper et al., 2015; Schnipper et al., 2016; Cherny et al., 2017). Both value frameworks aim to quantify the magnitude of value and reasonably assess affordable high-quality therapies for various cancer disease states (Kantarjian et al., 2013). Studies have shown that only one-third of positive trials meet the threshold for meaningful clinical benefit, and not all PD-1/PD-L1 inhibitors meet the threshold in the treatment of cancers (Del Paggio et al., 2017; Jiang et al., 2020).

Considering the inconsistencies in the evidence for PD-1/PD-L1 inhibitors in EC, GC and CRC and the challenge of increasing the tumor burden due to the skyrocketing price of new anti-tumor drugs, we carried out this study to quantify the value of PD-1/PD-L1 inhibitors in the treatment of EC, GC and CRC with ASCO-VF and ESMO-MCBS and to analysis the association between the value and cost of PD-1/PD-L1 inhibitors.

Methods

Selection of randomized controlled trials

We systematically searched eight databases, including Cochrane Library, PubMed, Embase, Web of Science (WOS), China National Knowledge Infrastructure (CNKI), Wanfang Data, Chongqing VIP (CQVIP), and Chinese BioMedical Literature Database (CBM), with the search terms "PD-1", "PD-L1", "gastric", "colorectal", "oesophageal" and "randomized controlled trial" to identify RCTs published from inception to 1 July 2022. The search strategy was preformulated by the research team and finally implemented by a team member (SL Ou). Furthermore, the reference lists of relevant systematic reviews were reviewed, and ClinicalTrials.gov was also checked to avoid omissions. Duplicate studies were removed by Endnote X9. The search strategy is detailed in Supplementary Table S1.

Studies were included that met the following criteria: 1) population: patients with EC, GC, gastroesophageal junction cancer (GEJC) and CRC; 2) intervention: PD-1/PD-L1 inhibitor monotherapy or in combination with chemotherapy (CT); 3) control: placebo or CT; 4) outcomes: hazard ratio (HR) of overall survival (OS), progression-free survival (PFS) or disease-free survival (DFS), grade 1–2 adverse events (AEs) and grade 3–4 AEs, quality of life (QoL); 5) study: Phase 2/3 RCT. Studies were excluded following exclusion criteria: 1) studies did not report survival curves or the rates of grade 1–2 AEs and grade 3–4 AEs; 2) non-Chinese or English literature.

Framework

The advanced disease and adjuvant or neoadjuvant therapy settings forms of ASCO-VF version 2 and ESMO-MCBS version 1.1 were used to assess the value scores (Schnipper et al., 2016; Cherny et al., 2017). ASCO-VF is designed for only in phase II or III RCT, including clinical benefit, toxicity and bonus points. The net health benefit (NHB) score is obtained by the final sum of the three module scores. The clinical benefit score is subtracted HR value the survival outcome indicator from 1, multiply by 100 points and then multiply by the weight (OS weighted 1, PFS weighted 0.8, ORR weighted 0.7). The toxicity score is the percentage difference between the total toxicity points of the intervention regimen and the control regimen multiply by 20 points. If the intervention regimen is more toxic than the control regimen, the toxicity score is subtracted from the clinical benefit score. If the toxicity of the intervention regimen was lower than the control regimen, the toxicity score is added to the clinical benefit score. Bonus points include 20 points for long-term survival (OS weighted 1, PFS weighted 0.8), 10 points for improvement in cancer-related symptoms, 10 points for quality of life, and percentage improvement in treatment-free interval multiply 20 points.

The ESMO-MCBS framework is designed for use only in positive trials, including clinical benefit, toxicity/quality of life. The clinical benefit grade is based on the lower limit of the 95% confidence interval (*CI)* of HR of survival outcome associated with a particular grade in a prespecified manner (e.g., grade 4 for control regimen with median OS $<$ 12 months, HR $\leq$ 0.65 and OS gain $\geq$3 months). Upgraded 1 level if improved quality of life or/and less specific 3–4 AEs are shown.

Finally, the net health benefit (NHB) scores of ASCO-VF are continuous data; ESMO-MCBS grades are distributed as 5, 4, 3, 2 or 1 for advanced disease setting and as A, B, or C for adjuvant or neoadjuvant therapy setting. ASCO-VF does not clearly define what score is considered the "meaningful value threshold", whereas ESMO-MCBS defines "meaningful clinical benefit" as a grade of 5, 4, A or B.

Data extraction and scoring

Two authors (SL Ou and JL) independently screened the titles and abstracts and full texts of eligible studies and used a standardized extraction form to extract the data. The extracted contents included the study name, phase, sample size, type of cancer, PD-1/PD-L1 inhibitors used, dosage regimen, follow-up time and outcomes. ASCO-VF scores and ESMO-MCBS grades were also independently evaluated by two authors (SL Ou and XL Qin). Any discrepancies were adjudicated by a third author (HW) to establish the final score or grade.

To assess the monthly cost of all anti-tumor drugs in the intervention and control groups of the included RCTs, we used the price of the branded name and generic drugs (often generic) from the Hospital Information System (HIS), which derived from the lowest wholesale pricing of the centralized procurement and drug price supervision platform of Sichuan Province and represented the actual purchase price of drugs in public medical institutions of the inter-provincial alliance. The monthly cost was calculated according to the dosage schedule in the included RCTs for a patient weighing 60 kg with a body surface area of 1.70 m^2. We reported the incremental monthly cost as the difference between the intervention and control groups. If the control group was placebo or best supportive care, the cost was set at zero. The most expensive one was recorded when the control group had multiple therapeutic regimens. The monthly cost of the therapeutic regimen was calculated over an average period of 30 days. Therapeutic regimens not available in China were not counted.

Statistical analysis

All data were collected using a standardized extraction form in an Excel file. Statistical analysis was performed with IBM SPSS (version 25.0). Continuous data were plotted to assess the normality of the underlying distribution. Comparisons between study groups were made using *Student's* t-test or the *Wilcoxon signed-ranked* test, as appropriate. We generated a receiver operating characteristic (ROC) curve to assess the predictive value of the ASCO-VF score in relation to the threshold of the ESMO-MCBS grade and evaluate the consistency of the two value frameworks. We used scatterplots and Pearson's or Spearman's correlation to show the association between incremental monthly cost and ASCO-VF scores or ESMO-MCBS grades. All analyses were deemed significant if $p < 0.05$.

Results

Study selection and characteristics

We identified 2086 records through initial retrieval. Ultimately, 33 studies reporting 23 RCTs published in English were considered eligible for this study (Kang et al., 2017; Bang et al., 2018; Shitara et al., 2018; Eng et al., 2019; Kato et al., 2019; Chen E. X. et al., 2020; André et al., 2020; Chen L. T. et al., 2020; Huang et al., 2020; Kojima et al., 2020; Shitara et al., 2020; Andre et al., 2021; Van Cutsem et al., 2021a; Boku et al., 2021; Van Cutsem et al., 2021b; Janjigian et al., 2021; Kelly et al., 2021; Luo et al., 2021; Moehler et al., 2021; Sun et al., 2021; Adenis et al., 2022; Antoniotti et al., 2022; Diaz et al., 2022; Doki et al., 2022; Fuchs et al., 2022; Kang et al., 2022; Lu et al., 2022; Mettu et al., 2022; Okada et al., 2022; Park et al., 2022; Shitara et al., 2022; Wang et al., 2022; Xu et al., 2022) (Figure 1). Of these, two (8.7%) RCTs were conducted in the setting of adjuvant therapy, while the others (91.3%) were conducted in the setting of advanced disease. Ten (43.48%) RCTs involved treatments for EC, five (21.74%) involved treatments for CRC, and eight (34.78%) involved treatments for GC/GEJC. Four (17.4%) RCTs had three arms, and the others (82.6%) had two arms. The median sample size was 493 (*IQR* 307–724), and all included studies were supported by pharmaceutical companies. More characteristics are presented in Table 1.

Value scores/grades

For the adjuvant therapy setting, durvalumab showed a negative value even compared with placebo, with an ASCO-VF score of −18.7. The application of ESMO-MCBS for nivolumab *versus* placebo resulted in a grade of A, which met the meaningful value threshold. For advanced diseases, all 25 therapeutic regimens met the evaluation criteria of ASCO-VF. The scores were normally distributed, ranging from −12.5 to 69. Since ASCO-VF has no clearly defined threshold for the meaningful value threshold, we used the mean score of 26.5 (95% *CI* 18.4–34.6) for subsequent analyses. Therefore, 12 (48%) regimens fell above the threshold, and 13 (52%) regimens fell below the threshold. The mean score of positive therapeutic regimens was 37.2 (95% *CI* 27.6–49.2), and the mean score of negative therapeutic regimens was 12.8 (95% *CI* 3.4–22.2). The value score of positive therapeutic regimens was significantly higher than that of negative therapeutic regimens ($p < 0.001$, *Student's* t-test). Fourteen positive therapeutic regimens met the evaluation criteria of ESMO-MCBS. Six (42.9%) of the regimens met the ESMO-MCBS benefit threshold grade, and eight (57.1%) of the regimens did not meet the ESMO-MCBS benefit threshold grade (Table 2).

The ROC curve was used to forecast the meaningful value threshold of ASCO-VF to meet the ESMO-MCBS in advanced disease. The threshold score was 38.2, which was close to that in our previous study (Jiang et al., 2020). Excitingly, the area under the curve was 1.0 ($p = 0.002$), suggesting exactly the same predictive value. Based on this result, ASCO-VF scores and ESMO-MCBS grades showed that pembrolizumab met the meaningful value threshold in the first-line treatment of EC and microsatellite instability–high CRC. Toripalimab and camrelizumab met meaningful value threshold in the first-line treatment of squamous cell EC, and nivolumab and camrelizumab met meaningful value threshold in second-line treatment. PD-1/PD-L1 inhibitors did not meet valuable threshold in GC/GEJC.

Correlation between value scores/grades and cost

The incremental monthly cost data of RCTs assessed by ASCO-VF were not normally distributed, thus, we analysed the correlation between value scores/grades and incremental monthly cost with Spearman's correlation. The incremental monthly cost and ASCO-VF scores were negatively correlated (Spearman's $\rho = -0.465$, $p = 0.034$, Figure 2). For ESMO-MCBS grades, the incremental monthly cost and value grades also showed a negative correlation (Spearman's $\rho = -0.211$, $p = 0.489$, Figure 3).

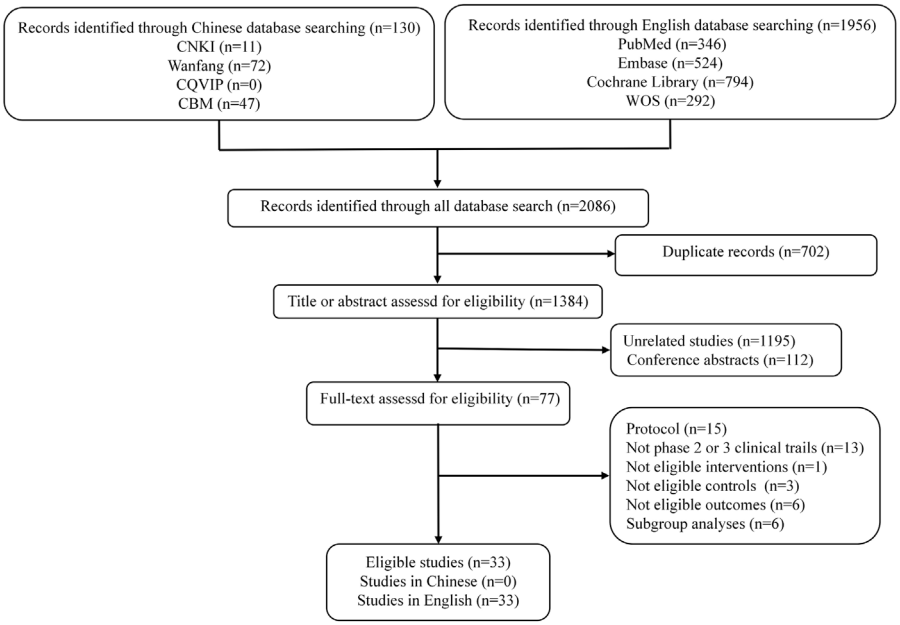

FIGURE 1
Flow diagram of study selection.

Discussion

Summary of results

The rising price of new anticancer drugs has led to public criticism of the pricing policies of manufacturers (Kantarjian et al., 2013). Coupled with the high burden of cancer, value assessment of new anti-tumor drugs has become an urgent need (Bach and Pearson, 2015). In this study, we assessed the value of PD-1/PD-L1 inhibitors in EC, GC and CRC using ASCO-VF and ESMO-MCBS. We found that only a few treatment regimens showed clinical value in EC and CRC. The association between ASCO-VF and ESMO-MCBS in this study was very well, and the value score/grade was negatively correlated with the incremental monthly cost.

Adjuvant chemotherapy after surgery is generally required for resectable locally advanced EC or GEJC. However, no treatment regimen has been shown to be effective, and the standard of care is best supportive care (Stahl et al., 2013; Ajani et al., 2019). In our study, nivolumab met valuable threshold in resectable locally advanced EC/GEJC (Kelly et al., 2021), which provides a new reference for clinical treatment and a new direction for clinical trials.

In regard to advanced diseases, 14 positive therapeutic regimens of 13 trials were assessed with both ASCO-VF and ESMO-MCBS, and 11 negative therapeutic regimens of 9 trials were assessed with only ASCO-VF. The NHB scores of positive trials were significantly higher than those of negative trials, and all negative trial scores were below the threshold predicted by the ROC curve. Considering that none of the 11 negative therapeutic regimens showed an improvement in QoL, we may conclude that a treatment is of no value when survival outcomes are not significantly increased while QoL is not improved, which is consistent with the use of ESMO-MCBS for non-inferiority (equivalence) studies (Cherny et al., 2015; Cherny et al., 2017). In GC/GEJC, none of the therapeutic regimens achieved the threshold value score or grade even when the PD-L1 combined positive score (CPS) was ≥1. PD-L1 inhibitor monotherapy or in combination with CT did not reach the threshold in CRC, but the PD-1 inhibitor pembrolizumab showed clinical value with an improvement in efficacy, toxicity and QoL as first-line therapy for microsatellite instability–high CRC (André et al., 2020; Andre et al., 2021; Diaz et al., 2022). In EC, pembrolizumab, toripalimab or camrelizumab in combination with CT showed clinical value in first-line treatment (Luo et al., 2021; Sun et al., 2021; Wang et al., 2022), and nivolumab and camrelizumab monotherapy showed value in second-line treatment (Kato et al., 2019; Huang et al., 2020; Okada et al., 2022). Although significant differences in survival outcomes have been at the forefront of drug approval and clinical decisions for many years, various stakeholders are increasingly focusing on the value (Vivot et al., 2017). In our study, we found that 8 of 14 positive therapeutic regimens did not meet the threshold value (Kang et al., 2017; Chen E. X. et al., 2020; Janjigian et al., 2021; Antoniotti et al., 2022; Doki et al., 2022; Lu et al., 2022; Xu et al., 2022), which suggests that the majority of positive interventions improved overall survival while compromising QoL or increasing the risk of toxicity. Therapeutic decisions should not be made solely on the $p < 0.05$ of survival indicators, and the clinical value of therapeutic regimens should be considered comprehensively.

Traditionally, we assume that the high price of new drugs is due to the need to support research; however, an analysis of transformative drugs shows that the main source of drug

TABLE 1 Characteristics of the included studies.

Registry number	Year	Study code	Phase	Disease type	Setting	Line	Intervention arm	Control arm	PD-L1 expression level	Sample size	Follow-up time m)	Industry sponsorship	Outcomes
NCT02520453 Park et al. (2022)	2022	—	II	EC (squamous carcinoma)	Adjuvant	—	Durvalumab	Placebo	—	86 (45/41)	38.7	Yes	OS, DFS, AEs
NCT02743494 Kelly et al. (2021)	2021	Checkmate 577	III	EC/GEJC	Adjuvant	—	Nivolumab	Placebo	—	894 (532/262)	24.4	Yes	DFS, AEs
NCT02873195 Mettu et al. (2022)	2022	BACCI	II	CRC	Advanced	2	Atezolizumab + Bevacizumab + Capecitabine	Placebob + Bevacizumab + Capecitabine	—	133 (86/47)	20.9	Yes	OS, PFS, ORR, AEs
NCT02563002 André et al. (2020); Andre et al. (2021); Diaz et al. (2022)	2020	KEYNOTE-177	III	CRC	Microsatellite instability–high advanced	1	Pembrolizumab	Oxaliplatin + Leucovorin+5-fluoropyrimidine + Bevacizumab or Cetuximab	—	307 (153/154)	44.5	Yes	PFS, ORR, AEs
NCT02788279 Eng et al. (2019)	2019	IMblaze370	III	CRC	Advanced	3	Atezolizumab + Cobimetinib Atezolizumab	Regorafenib	—	363 (183/ 90/90)	7.3	Yes	OS, PFS, ORR, AEs
NCT03721653 Antoniotti et al. (2022)	2022	Atezo TRIBE	II	CRC (adenocarcinoma)	Advanced	1	Atezolizumab + Bevacizuma + Irinotecan + Oxaliplatin + Leucovorin+5-fluoropyrimidine	Bevacizuma + Irinotecan + Oxaliplatin + Leucovorin+5-fluoropyrimidine	—	218 (145/73)	19.9	Yes	PFS, ORR, AEs
NCT02870920 Chen et al. (2020a)	2020	—	II	CRC (adenocarcinoma)	Advanced	≥3	Durvalumab + Tremelimumab + Best supportive care	Best supportive care	—	180 (119/61)	15.2	Yes	OS, PFS, AEs
NCT02564263 Kojima et al. (2020); Adenis et al. (2022)	2020	KEYNOTE-181	III	EC	Advanced	2	Pembrolizumab	Paclitaxel or Docetaxel or Irinotecan	—	628 (314/314)	11.1	Yes	OS, PFS, ORR, AEs
	2021												
NCT03189719 Sun et al. (2021)	2021	KEYNOTE-590	III	EC	Advanced	1	Pembrolizumab+5-fluoropyrimidine + Cisplatin	Placebo+5-fluoropyrimidine + Cisplatin	—	749 (373/376)	22.6	Yes	OS, PFS, ORR, AEs
NCT03116152 Xu et al. (2022)	2022	ORIENT-2	II	EC (squamous carcinoma)	Advanced	2	Sintilimab	Paclitaxel or Irinotecan	—	190 (95/95)	7.2	Yes	OS, PFS, ORR, AEs
NCT03143153 Doki et al. (2022)	2022	CheckMate 648	III	EC (squamous carcinoma)	Advanced	1	Nivolumab+5-fluoropyrimidine + Cisplatin Nivolumab + Ipilimumab	5-fluoropyrimidine + Cisplatin	—	970 (321/ 325/324)	13	Yes	OS, PFS, ORR, AEs
NCT03748134 Lu et al. (2022)	2022	ORIENT-15	III	EC (squamous carcinoma)	Advanced	1	Sintilimab+(Cisplatin + Paclitaxel) or (5-fluoropyrimidine + Cisplatin)	Placebo+(Cisplatin + Paclitaxel) or (5-fluoropyrimidine + Cisplatin)	—	659 (327/332)	16.9	Yes	OS, PFS, ORR, AEs
NCT03829969 Wang et al. (2022)	2022	JUPITER-06	III	EC (squamous carcinoma)	Advanced	1	Toripalimab + Cisplatin + Paclitaxel	Cisplatin + Paclitaxel	—	514 (257/257)	7.1	Yes	OS, PFS, ORR, AEs
NCT03691090 Luo et al. (2021)	2021	ESCORT-1	III	EC (squamous carcinoma)	Advanced	1	Camrelizumab + Cisplatin + Paclitaxel	Placebo + Cisplatin + Paclitaxel	—	596 (298/298)	10.8	Yes	OS, PFS, ORR, AEs

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TABLE 1 (*Continued*) Characteristics of the included studies.

Registry number	Year	Study code	Phase	Disease type	Setting	Line	Intervention arm	Control arm	PD-L1 expression level	Sample size	Follow-up time m)	Industry sponsorship	Outcomes
NCT03099382 Huang et al. (2020)	2020	ESCORT	III	EC (squamous carcinoma)	Advanced	2	Camrelizumab	Docetaxel or Irinotecan	—	457 (229/228)	8.3	Yes	OS, PFS, ORR, AEs
NCT02569242 Kato et al. (2019); Okada et al. (2022)	2019	ATTRACTION-3	III	EC (squamous carcinoma)	Advanced	2	Nivolumab	Paclitaxel or Docetaxel	—	419 (210/209)	36	Yes	OS, PFS, ORR, AEs
NCT02872116 Janjigian et al. (2021); Shitara et al. (2022)	2021	CheckMate 649	III	GC/EC/GEJC (adenocarcinoma)	Advanced	1	Nivolumab+(Capecitabine + Oxaliplatin) or (Oxaliplatin + Leucovorin+5-fluoropyrimidine) Nivolumab + Ipilimumab	(Capecitabine + Oxaliplatin) or (Oxaliplatin + Leucovorin+5-fluoropyrimidine)	—	2031 (789/792/450)	24	Yes	OS, PFS, ORR, AEs
NCT02746796 Kang et al. (2022)	2022	ATTRACTION-4	III	GC/GEJC	HER2-negative advanced	1	Nivolumab + Capecitabine + Oxaliplatin	Placebo + Capecitabine + Oxaliplatin	—	724 (362/362)	26.5	Yes	OS, PFS, ORR, AEs
NCT02625623 Bang et al. (2018)	2018	JAVELIN Gastric 300	III	GC/GEJC	Advanced	3	Avelumab	Paclitaxel or Irinotecan	—	371 (185/186)	10.6	Yes	OS, PFS, ORR, AEs
NCT02267343 Kang et al. (2017); Chen et al. (2020b); Boku et al. (2021)	2017	ATTRACTION-2	III	GC/GEJC	Advanced	3	Nivolumab	Placebo	—	493 (330/163)	36	Yes	OS, PFS, ORR, AEs
NCT02494583 Shitara et al. (2020); Van Cutsem et al. (2021b)	2020	KEYNOTE-062	III	GC/GEJC (adenocarcinoma)	Advanced	1	Pembrolizumab Pembrolizumab + Cisplatin or Capecitabine	Placebo + Cisplatin or Capecitabine	PD-L1 CPS≥1	763 (256/257/250)	29.4	Yes	OS, PFS, ORR, AEs
NCT02625610 Moehler et al. (2021)	2020	JAVELIN Gastric 100	III	GC/GEJC (adenocarcinoma)	Advanced	1	Avelumab	Oxaliplatin + Leucovorin+5-fluoropyrimidine	—	499 (249/250)	24	Yes	OS, PFS, ORR, AEs
NCT02370498 Shitara et al. (2018); Van Cutsem et al. (2021a); Fuchs et al. (2022)	2018	KEYNOTE-061	III	GC/GEJC (adenocarcinoma)	Advanced	2	Pembrolizumab	Paclitaxel	PD-L1 CPS≥1	395 (196/199)	52	Yes	OS, PFS, ORR, AEs

Note: EC, oesophageal cancer; GC, gastric cancer; GEJC, gastroesophageal junction cancer; CPS, combined positive score; OS, overall survival; PFS, progression-free survival; ORR, objective response rate; DFS, disease-free survival; AEs, adverse events; /, not reported.

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TABLE 2 Clinical benefit according to ASCO-VF and ESMO-MCBS.

Registry number	Intervention arm	Primary outcome	Primary outcome HR (95% *CI*)	ASCO-VF				ESMO-MCBS			Monthly incremental cost (¥)
				Clinical benefit score	Toxicity score	Bonus points	NHB	Clinical benefit grade	Quality of life/ Grade 3–4 toxicities	ESMO-MCBS	
NCT02520453 Park et al. (2022)	Durvalumab	OS	1.08 (0.52–2.24)	−8	−10.7	0	−18.7	NA	NA	NA	51756.92
NCT02743494 Kelly et al. (2021)	Nivolumab	DFS	0.69 (0.56–0.86)	31	−10	0	21	A	0	A	49471.59
NCT02873195 Mettu et al. (2022)	Atezolizumab + CT	OS	0.96 (0.63–1.45)	4	−2	0	2	NA	NA	NA	46857.14
NCT02563002 André et al. (2020); Andre et al. (2021); Diaz et al. (2022)	Pembrolizumab	PFS	0.59 (0.45–0.79)	32.8	13.6	20	66.4	3	1	4	24579.4
NCT02788279 Eng et al. (2019)	Atezolizumab + Cobimetinib	OS	1.00 (0.73–1.38)	0	−0.4	0	−0.4	NA	NA	NA	—
	Atezolizumab	OS	1.19 (0.83–1.71)	−19	6.5	0	−12.5	NA	NA	NA	26159.54
NCT03721653 Antoniotti et al. (2022)	Atezolizumab + CT	PFS	0.69 (0.56–0.85)	24.8	−5.3	0	19.5	2	0	2	70285.71
NCT02870920 Chen et al. (2020a)	Durvalumab + Tremelimumab	OS	0.72 (0.54–0.97)	28	−5.9	0	22.1	3	0	3	—
NCT02564263 Kojima et al. (2020); Adenis et al. (2022)	Pembrolizumab	OS	0.89 (0.75–1.05)	11	20	0	31	NA	NA	NA	39537.14
NCT03189719 (Sun et al. (2021)	Pembrolizumab + CT	OS	0.73 (0.62–0.86)	27	−1.9	20	45.1	4	0	4	51194.29
NCT03116152 Xu et al. (2022)	Sintilimab	OS	0.70 (0.50–0.97)	30	7.5	0	37.5	1	1	2	−8571.43
NCT03143153 Doki et al. (2022)	Nivolumab + CT	OS	0.74 (0.58–0.96)	26	−3.1	0	22.9	3	0	3	49471.59
	Nivolumab + Ipilimumab	OS	0.78 (0.62–0.98)	22	9	0	31	3	0	3	79478.89
NCT03748134 Lu et al. (2022)	Sintilimab + CT	OS	0.63 (0.51–0.78)	37	−1.8	0	35.2	3	0	3	3085.71
NCT03829969 Wang et al. (2022)	Toripalimab + CT	OS	0.58 (0.43–0.78)	42	−3.2	0	38.8	4	0	4	2732.8
NCT03691090 Luo et al. (2021)	Camrelizumab + CT	OS	0.70 (0.56–0.88)	30	−1.9	20	48.1	3	1	4	4182.86
NCT03099382 Huang et al. (2020)	Camrelizumab	OS	0.71 (0.57–0.87)	29	20	20	69	3	1	4	−5382.86
NCT02569242 Kato et al. (2019); Okada et al. (2022)	Nivolumab	OS	0.79 (0.64–0.97)	21	17.5	10	48.5	3	1	4	47989.56
NCT02872116 Janjigian et al. (2021); Shitara et al. (2022)	Nivolumab + CT	OS	0.79 (0.71–0.88)	21	−1.7	0	19.3	2	0	2	49471.59

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TABLE 2 (*Continued*) Clinical benefit according to ASCO-VF and ESMO-MCBS.

Registry number	Intervention arm	Primary outcome	Primary outcome HR (95% *CI*)	ASCO-VF				ESMO-MCBS			Monthly incremental cost (¥)
				Clinical benefit score	Toxicity score	Bonus points	NHB	Clinical benefit grade	Quality of life/ Grade 3–4 toxicities	ESMO-MCBS	
	Nivolumab + Ipilimumab	OS	0.91 (0.77–1.07)	9	3.2	0	12.2	NA	NA	NA	173104.97
NCT02746796 Kang et al. (2022)	Nivolumab + CT	OS	0.90 (0.75–1.08)	10	−1.3	0	8.7	NA	NA	NA	52638.51
NCT02625623 Bang et al. (2018)	Avelumab	OS	1.11 (0.90–1.40)	−11	15.6	0	4.6	NA	NA	NA	—
NCT02267343 Kang et al. (2017); Chen et al. (2020b); Boku et al. (2021)	Nivolumab	OS	0.62 (0.50–0.75)	38	−20	0	18	1	0	1	39478.89
NCT02494583 Shitara et al. (2020); Van Cutsem et al. (2021b)	Pembrolizumab	OS	0.91 (0.69–1.18)	9	20	0	29	NA	NA	NA	51194.29
	Pembrolizumab + CT	OS	0.85 (0.70–1.03)	15	0.2	0	15.2	NA	NA	NA	50511.43
NCT02625610 Moehler et al. (2021)	Avelumab	OS	0.91 (0.74–1.11)	9	20	0	29	NA	NA	NA	—
NCT02370498 Shitara et al. (2018); Van Cutsem et al. (2021a); Fuchs et al. (2022)	Pembrolizumab	OS	0.81 (0.66–1.00)	19	2.8	0	21.8	NA	NA	NA	50113.64

Note: CT, chemotherapy; OS, overall survival; PFS, progression-free survival; DFS, disease-free survival; NHB, net health benefit; NA, not applicable; /: not available in China.

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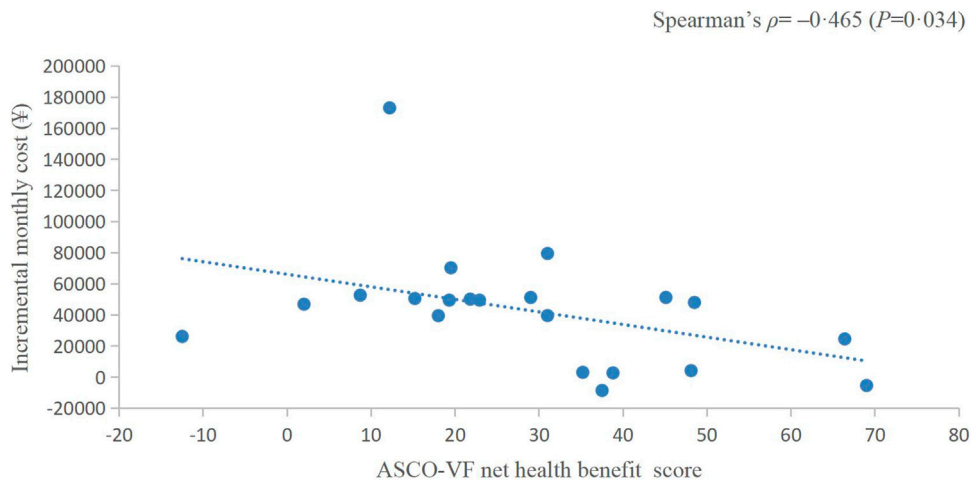

FIGURE 2
Scatterplot of the correlation between ASCO-VF net health benefit scores and incremental monthly cost.

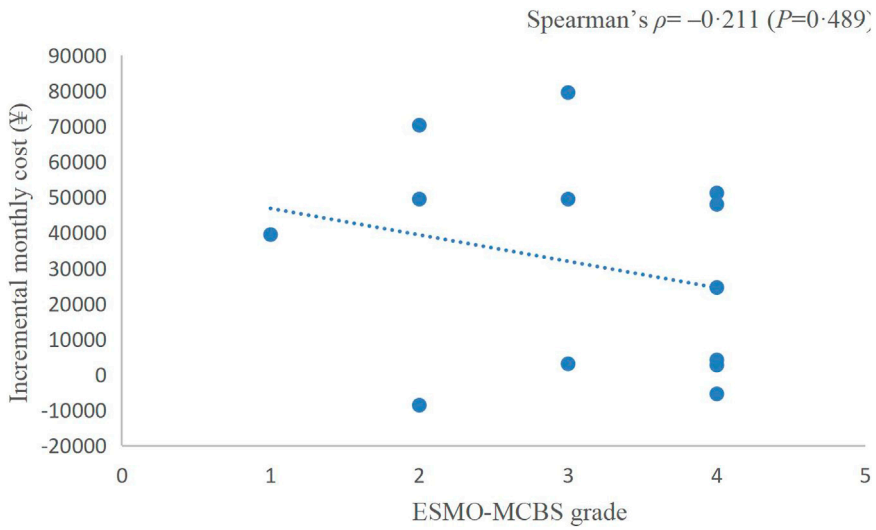

FIGURE 3
Scatterplot of correlation between ESMO-MCBS grades and incremental monthly cost.

innovation is government-funded academic research (Kesselheim et al., 2015). As the payer of medical activities, the price paid by patients for drugs should have a positive relationship with the value created. In recent years, a series of studies have shown that there is no statistically significant association between the value and prices of anticancer drugs (Vivot et al., 2017; Jiang et al., 2020; Vokinger et al., 2020). Interestingly, in this study, we found a negative correlation between the prices of PD-1/PD-L1 inhibitors and their value. This negative correlation between prices and the ASCO-VF value score was even statistically significant (Spearman's $\rho = -0.465$, $p = 0.034$), resulting in an urgent demand for value-based pricing. Camrelizumab and toripalimab showed clinical value in EC and have relatively low prices in the Chinese market, so their value may be more worthy of payment, which was also consistent with the results of China's national price negotiations (Zhang et al., 2022).

Implications

This study has some implications. Firstly, this study shows no clinical value for PD-1/PD-L1 inhibitors in GC/GEJC, which suggests that subsequent clinical trials on the treatment of GC/GEJC with PD-1/PD-L1 inhibitors should fully follow the current evidence. Secondly, the prices of PD-1/PD-L1 inhibitors are not aligned with their value. Price negotiation for higher-priced PD-1/PD-L1 inhibitors should be prioritized to improve patient access to beneficial drugs, thereby contributing to patient-

centred cancer treatment goals. Thirdly, all therapeutic regimens with improved QoL showed clinical value (Kato et al., 2019; Huang et al., 2020; Luo et al., 2021; Sun et al., 2021), suggesting that clinical trials and clinical treatment strategies should pay more attention to QoL.

Limitations

We comprehensively assessed the value of PD-1/PD-L1 inhibitors in oesophageal and gastrointestinal cancer with ASCO-VF and ESMO-MCBS, and we acknowledged some limitations. Firstly, the number of RCTs included in this study was small, and there were only 14 therapeutic regimens that met both the ASCO-VF and ESMO-MCBS criteria. Although the consistency of the two value frameworks in this study was perfect, the conclusion may exist the risk of bias due to the influence of the small sample size. Secondly, as ASCO-VF did not define toxicity scores for subgroup analyses, they could not be evaluated in the subgroup analyses. Therefore, the subgroup results of PD-L1 expression and microsatellite stability level were partially incomplete. Thirdly, we used the pricing system of public hospitals and centralized procurement and drug price supervision platforms of Sichuan province in China, so the results of the correlation between the value scores/grades and cost do not necessarily apply to countries outside of China. Finally, we only considered drug costs when calculating monthly increments, without taking into account the patients and their spouses or other important people due to absence, emergency treatment, hospitalization and medical expenses. In fact, because these costs are not easy to obtain directly, value frameworks consider only the cost of drugs as a rough estimate of the cost of treatment.

Conclusion

ASCO-VF and ESMO-MCBS could identify therapeutic regimens with clinical value. The incremental monthly cost for PD-1/PD-L1 inhibitors was not proportional to their value. PD-1/PD-L1 inhibitors did not meet valuable threshold in GC/GEJC. Pembrolizumab met the valuable threshold in advanced microsatellite instability–high CRC. The value of camrelizumab and toripalimab may be more worth paying in EC.

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

S-LO and QJ designed the study and rigorously drafted and revised the manuscript for important intellectual content. JL, X-LQ, HW, and S-LO conducted the literature search and data extraction. S-LO analysed and interpreted the data. All authors read and approved the final 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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Supplementary material

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The safety concerns regarding immune checkpoint inhibitors in liver cancer patients rising mainly from CHB

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Aim: To analyze the safety of immune checkpoint inhibitors in primary liver cancer patients and to identify the risk factors for immune-related adverse events (irAEs).

Methods: The study enrolled 106 patients with primary liver cancer, including 81 with hepatocellular carcinoma and 25 with intrahepatic cholangiocarcinoma. We analyzed the differences between groups in irAE occurrence, including those with and without targeted drugs and those who received interventional therapy.

Results: The incidence of irAEs was 39%, with thyroid function, liver function, and skin events being the most common. There was no correlation among irAE incidence and the liver cancer type, stage, or severity; grade of Child–Pugh score; and Barcelona Clinical Liver Cancer classification. However, being overweight was a significant risk factor for irAEs, correlating with high body mass index. The combination of targeted drugs and/or transcatheter arterial chemoembolization therapy did not increase the incidence of irAEs.

Conclusion: Being overweight is a potential risk factor for irAEs in primary liver cancer patients. However, there is no correlation between irAE incidence and the liver cancer type, stage, or severity or a combination of targeted drugs or transarterial chemoembolization therapy.

KEYWORDS

primary liver cancer, immune checkpoint inhibitor, immunotherapy, combined therapy, immune-related adverse events

Abbreviations: HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; irAE, immune-related adverse reactions; TACE, transarterial chemical embolization; ICIs, immune checkpoint inhibitors; HBV, hepatitis B virus; HCV, hepatitis C virus; PD-1, programmed death-1 receptor; PD-L1, programmed death-ligand 1; CTLA-4, cytotoxic T-lymphocyte antigen 4; TKI, tyrosine kinase inhibitors; BCLC, Barcelona Clinical Liver Cancer; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AKP, alkaline phosphatase; GGT, glutamyl transferase; TB, total bilirubin; BMI, body mass index.

Introduction

Primary liver cancer is a prevalent cancer type globally, with China accounting for more than half of all cases and a continuing increase in morbidity rates (Chen et al., 2016). Despite efforts toward early detection, a significant proportion of liver cancer patients are diagnosed at advanced stages, leading to unacceptably poor 5-year survival rates of only 12.5% (Zeng et al., 2018). The poor prognosis is mainly due to patients presenting with metastasis at the initial diagnosis, thereby losing the chance for surgical resection or liver transplantation (Bruix and Sherman, 2011; Labib et al., 2017). While local treatments such as radiofrequency ablation (RFA) and transarterial chemoembolization (TACE) are alternative approaches, they have limited benefits (Li and Ni, 2019).

More recently, immune checkpoint inhibitors (ICIs) have emerged as a promising approach in the management of malignant tumors (Sharon et al., 2014). ICIs work by blocking pathways that lead to T-cell inactivation and promote tumor cell death. The most impressive outcomes of ICIs have been observed in melanoma and non-small-cell lung cancer (Smyth et al., 2016; Dafni et al., 2019; Wang et al., 2019; Chang et al., 2020). However, the successful outcomes of ICIs are compromised due to some serious immune-related adverse events (irAEs) in the skin and thyroid (Xu et al., 2019; Qin et al., 2020). Furthermore, there is a relatively high incidence of irAEs detected from liver cancer patients following the administration of ICIs, although the efficacy of ICIs has also been observed.

It is crucial to acknowledge that although ICIs have demonstrated great potential in treating specific types of cancers, not all patients respond to this treatment equally well (Khoja et al., 2017). Several factors such as the cancer stage and type, a patient's overall health and age, and comorbidities can all influence their response to ICIs. Therefore, it is necessary to customize the treatment approach for each patient, taking into account the potential advantages and disadvantages of combining various treatments.

Therefore, the combination of ICIs with other anti-cancer agents or with other ICIs is being explored to improve treatment efficacy with promising results (Ruf et al., 2021). However, it remains unclear whether combining immunosuppressive therapy with tyrosine kinase inhibitors and interventional therapy is safe for primary liver cancer patients, necessitating further research in this area. As such, we conducted a retrospective study to compare the incidence of irAEs between patients with liver cancer receiving ICI monotherapy and those receiving combined immunotherapy (TACE and/or TKI).

Patients and methods

Study design

This retrospective study was conducted at Ruijin Hospital, Shanghai, China, and included patients with primary liver cancer, including hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC), who received anti-PD-1/PD-L1 treatment between July 2019 and July 2021. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board and Human Ethics Committee of Ruijin Hospital. Written informed consent was waived due to the retrospective nature of the study.

Patients

The patients included in this retrospective study met the following inclusion criteria: 1) a diagnosis of HCC or ICC confirmed by radiological or pathological findings according to the AASLD practice guidelines (Heimbach et al., 2018); 2) received at least one dose of ICIs, including PD-1 inhibitors (sintilimab, camrelizumab, pembrolizumab, tislelizumab, toripalimab, and nivolumab) or PD-L1 inhibitors (atezolizumab). Patients were excluded if they met the following criteria: 1) a history of previous ICI treatment; 2) an active or silent malignant tumor other than HCC or ICC; 3) a previous diagnosis of autoimmune disease; 4) severe cardiovascular disease (including unstable angina pectoris); 5) a serious infection; 6) a history of allergy to related drugs; and 7) pregnancy or lactation. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board and Human Ethics Committee of Ruijin Hospital. Written informed consent was waived due to the retrospective nature of the study.

Data collection

We collected the following clinical and laboratory information from the electronic health system records of Ruijin Hospital: age; sex; body mass index (BMI); etiology and severity of the liver disease; absence or presence of cirrhosis; assessment of liver cancer, including the size and number of tumors, vascular invasion, and extrahepatic metastasis on imaging; stages of Barcelona Clinical Liver Cancer (BCLC); Eastern Cooperative Oncology Group performance status (ECOG PS); and treatment information on the etiology of liver disease and HCC or ICC, including PD-1 inhibitors (sintilimab, camrelizumab, pembrolizumab, tislelizumab, toripalimab, and nivolumab), TKIs, and TACE. In addition, we collected laboratory data including alpha-fetoprotein (AFP), protein induced by vitamin K absence or antagonist-II (PIVKA-II), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), glutamyl transferase (GGT), albumin (ALB), total bilirubin (TBIL), prothrombin time (PT), blood lipid levels (triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and glucose (GLU)), and electrolyte levels (potassium (K), sodium (Na), chloride (Cl), calcium (Ca), and phosphorus (P)). Additionally, we collected serum markers of hepatitis virus infection, including HBeAg and anti-HBS, anti-HBC, anti-HBE, anti-HCV antibodies, serum HBV DNA levels, and serum HCV RNA levels.

Assessment of immune-related adverse events

The assessment of immune-related adverse events was conducted using the National Cancer Institute (NCI) Common Terminology for Adverse Events (CTCAE v.4.03) and was graded at each visit. Follow-up was performed every 3 weeks after the first dose of ICI until July 2021 by two experienced infectious disease physicians. irAEs, including rash, abdominal pain, diarrhea, ocular symptoms, myocardial enzymes, thyroid function, glucocorticoid

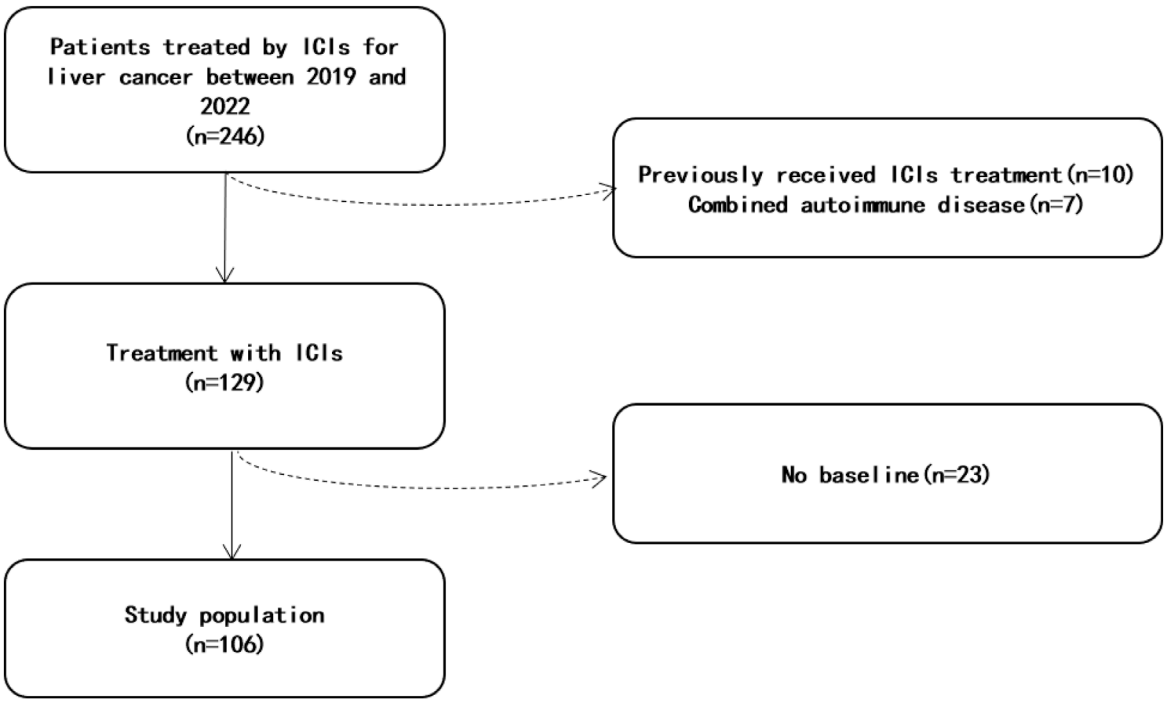

FIGURE 1
Flowchart.

levels, ACTH hormone levels, liver function, renal function, blood glucose and lipids, and chest CT images, were observed.

Statistical analysis

The statistical analysis was performed using SPSS Statistics 26. Normally, the distributed measurement data were presented as mean ± standard deviation (SD), and one-way ANOVA was used to compare multiple groups. Non-normally distributed data were described using median and quartile spacing, and the Kruskal–Wallis test was used to compare mean values between multiple groups. The frequency (composition ratio) and kappa test were used to describe classification variables. A two-sided p-value <0.05 was considered statistically significant.

Results

Patient characteristics

This study included a total of 106 liver cancer patients treated with anti-PD-1 therapy (Figure 1). The median age was 59 years (range 27–82), and the majority of them were male (85%) (Table 1). Chronic liver disease was present in 71% of patients, with HBV being the most common etiology. Most patients (68%) had Child–Pugh grade A liver function. The majority of patients (66%) were BCLC stage C, with 13%, 16%, and 6% being stages A, B, and D, respectively. Prior to anti-PD-1 therapy, 79% of patients had received targeted therapy, 69% had undergone TACE, and 43% had liver cancer resection surgery. Only 9% of ICC patients had chemotherapy. The average number of cycles of anti-PD-1 treatment for all patients was 4.25 ± 3.80. At the final investigation, 45 patients (42%) were still receiving PD-1 treatment, while six patients died due to liver cancer progression (five) and septic shock (one).

A comparison of baseline data between patients with and without irAEs (n = 44 and n = 62, respectively) was performed (Table 2). The irAE group had a BMI higher than that of the non-irAE group (23.73 *vs.* 22.55; $p = 0.037$) (Figure 2). The proportion of overweight patients (BMI ≥24 kg/m^2) in the irAE group was higher than that in the non-irAE group (39% *vs.* 21%; $p = 0.047$). No significant differences were observed between the two groups in terms of sex, age, liver basic condition, combination of drugs, basic metabolic diseases, liver function, electrolytes, or other factors.

Safety of PD-1 inhibitors

The safety of PD-1 inhibitors was evaluated, with a summary of the types, incidence, and average time of immune-related adverse events during ICI treatment (Figure 3). Among the 106 liver cancer patients, 44 (38%) experienced irAEs, with 14 (13%) being grade 3/4. The observed irAE subtypes were thyroid (15%), liver (13%), skin (10%), diabetes (1 patient), and hyperlipidemia (1 patient), with 15% of the patients experiencing two or more subtypes. Only one HCC patient (0.94%) experienced delayed irAEs with adrenal hypofunction 12 months after treatment discontinuation. Out of the 44 patients with irAEs, 33 (75%) were HCC patients and 11 (25%) were ICC patients (Supplementary Table S1), with no significant difference in irAE incidence between the two groups. Looking at it from another angle, 33/81 (40%) HCC patients or 11/25 (44%) ICC patients had irAEs (Figure 4). Additionally, there was no significant difference in irAE incidence

TABLE 1 Baseline characteristics of patients with liver cancer treated with ICIs.

	Total (n = 106)
Age (years)	59 ± 11
Sex	
Male, n (%)	85 (80%)
Female, n (%)	21 (20%)
Underlying liver disease, n (%)	
HBV	75 (71%)
HCV	3 (3%)
Unknown	28 (27%)
Basal metabolic disease	
Hypertension	39 (37%)
Hyperlipidemia	7 (7%)
Diabetes	26 (25%)
BMI (kg/m^2)	23 ± 3
AFP (ng/mL)	1,555 ± 5,374
CA19-9 (U/mL)	670 ± 2,246
Cirrhosis, n (%)	69 (65%)
Ascites, n (%)	27 (26%)
Vascular invasion, n (%)	42 (40%)
Extrahepatic metastasis, n (%)	48 (45%)
Postoperative recurrence, n (%)	27 (25%)
ECOG PS, n (%)	
0	101 (95%)
≥1	5 (5%)
Child–Pugh, n (%)	
A	72 (68%)
B	29 (27%)
C	1 (1%)
Unknown	4 (4%)
BCLC stage, n (%)	
A	13 (12%)
B	17 (16%)
C	70 (66%)
D	6 (6%)
Prior treatment, n (%)	
Surgical resection	44 (42%)
TACE	73 (69%)
TKI	84 (80%)
Chemotherapy	9 (9%)

among liver cancer patients who received ICI therapy alone, combined targeted drugs, or combined TACE therapy.

Regarding liver irAEs during ICI therapy, 14 (13%) patients developed full-grade hepatitis, with 10 (9%) having grade 3–4 (Table 3). There were no deaths due to irAEs. Liver adverse events were mainly increased GGT (n = 10) and ALP (n = 8), followed by TB (n = 5). Among these patients, increased AST (n = 4) and ALT (n = 2) were relatively rare. Out of the 14 patients, 11/81 (14%) were HCC patients and 3/25 (12%) were ICC patients. There were 11/78 (15%) patients with underlying hepatic diseases (HBV and HCV) and 3/28 (11%) patients without underlying hepatic diseases. The incidence of hepatic adverse events in patients with underlying hepatic diseases was slightly higher than in those without underlying hepatic diseases.

Regarding the earliest occurrence time of irAEs, we further analyzed the four groups of patients with irAEs (Table 4). There was a significant difference between PD-1 alone (23 ± 3) and PD-1/TACE (52 ± 79) groups (p = 0.006). PD-1/TKI was significantly different from PD-1/TACE/TKI (35 ± 32 *vs.* 95 ± 95; p = 0.05). Further grouping analysis showed that TKI and TACE combination treatment did not affect the earliest occurrence time of irAEs (78 ± 87 *vs.* 78 ± 83), while TACE combination treatment delayed the earliest occurrence time of irAEs (100 ± 94 *vs.* 31 ± 27; $p < 0.0001$).

Subgroup analysis: patients with HBV infections

Out of the 75 liver cancer patients, 64 (85%) were HBsAg positive, with only 47 of these CHB patients having HBsAg quantification (mean value 982 ± 2213 IU/mL). Among the 71 CHB patients with HBV DNA quantification, 55 had HBV DNA levels <2 × 103 IU/mL and 16 had 2 × 103 to 2 × 107 IU/mL. Out of the 75 CHB patients, 48 (64%) had received prior antiviral therapy, 18 (24%) had simultaneous antiviral and PD-1 treatment, and 9 (12%) had PD-1 treatment only. No HBV reactivation was observed during the treatment.

The change in HBsAg levels of 31 HBsAg+ patients was collected at the end of the follow-up period, showing a ~70% reduction from baseline to the end of the follow-up period (1,257 ± 2,606 *vs.* 504 ± 964; $p > 0.05$) (Supplementary Figure S1). These CHB patients were further categorized into three groups based on their baseline HBsAg levels (i.e., HBsAg<100 IU/mL, 100 IU/mL < HBsAg <1000 IU/mL, or HBsAg >1000 IU/mL groups). The change in HBsAg levels from baseline to the end of ICI treatment was 35 ± 26 *vs.* 36 ± 31 IU/mL ($p > 0.05$), 340 ± 297 IU/mL *vs.* 238 ± 270 IU/mL (p = 0.019), and 3,340 ± 3,592 IU/mL *vs.* 1,262 ± 1,328 IU/mL ($p > 0.05$) in HBsAg <100 IU/mL, 100 < HBsAg <1000 IU/mL, and HBsAg >1000 IU/mL groups, respectively.

Discussion

Patients with HBV-associated liver tumors are at a high risk of HBV reactivation, which can result in poor overall survival outcomes (Jang, 2014). ICIs have been reported to cause HBV reactivation with varying outcomes, ranging from full recovery to liver failure. The mechanism behind HBV reactivation induced by ICIs is not fully understood, but studies have suggested that ICIs may promote Treg proliferation, disrupt immune homeostasis, or lead to the release of previously dormant viruses into circulation (Keir et al., 2008; Franceschini et al., 2009; Knolle and Thimme, 2014; Cho et al., 2017). A lack of antiviral prophylaxis has been identified as a significant factor in HBV reactivation, with some studies reporting a reactivation rate of 5.3% in HBsAg+ patients (Yoo et al., 2021). However, other studies have shown lower rates of reactivation in HBsAg+ patients (1.0%) and no reactivation in HBsAg– patients, regardless of the HBcAb status (Yoo et al., 2021).

The majority of patients in the present study were HBV positive (HBsAg$^{+/-}$) and had received antiviral therapy before the initiation of the ICI treatment, and no HBV reactivation was detected. Moreover, concurrent antiviral therapy with ICIs did not lead to HBV reactivation, emphasizing the significance of antiviral prophylaxis in preventing HBV reactivation in immunosuppressed patients (Lee et al., 2020; Yoo et al., 2021; Zhao et al., 2022).

TABLE 2 Univariate analyses of the factors associated with irAEs.

	irAE (n = 44)	Non-irAE (n = 62)	*p*-value
Sex			NS
Male, n (%)	33 (75%)	52 (84%)	
Female, n (%)	11 (25%)	10 (16%)	NS
Age	58 ± 11	60 ± 11	NS
Cirrhosis, n (%)	29 (66%)	40 (65%)	NS
Basal metabolic disease, n (%)	33 (75%)	45 (73%)	NS
Extrahepatic metastasis, n (%)	23 (52%)	25 (40%)	NS
Hypertension, n (%)	17 (39%)	22 (35%)	NS
Hyperlipidemia, n (%)	4 (9%)	3 (5%)	NS
Diabetes, n (%)	10 (23%)	16 (26%)	NS
BMI (kg/m^2)	23.7 ± 3.7	22.6 ± 3.0	0.037
Glucose (mmol/L)	5.5 ± 1.7	6.1 ± 2.4	NS
Triglyceride (mmol/L)	1.1 ± 0.3	1.0 ± 0.3	NS
Total cholesterol (mmol/L)	4.1 ± 1.0	4.2 ± 1.0	NS
Free fatty acid (mmol/L)	0.6 ± 0.3	0.6 ± 0.3	NS
PLT (10^9/L)	139.2 ± 79.8	151.2 ± 88.7	NS
ALT (IU/L)	40.4 ± 30.q	38.0 ± 25.5	NS
AST (IU/L)	55.6 ± 44.3	57.3 ± 40.4	NS
AKP (IU/L)	171.4 ± 117.4	170.6 ± 90.2	NS
GGT (IU/L)	140.9 ± 111.4	163.4 ± 119.5	NS
TBIL (μmol/L)	20.6 ± 10.5	29.3 ± 35.6	NS
ALB (g/L)	35.3 ± 5.2	35.1 ± 5.3	NS
TBA (μmol/L)	16.6 ± 17.8	20.1 ± 28.1	NS
Na (mmol/L)	139.1 ± 3.9	138.6 ± 3.8	NS
K (mmol/L)	4.0 ± 0.5	4.0 ± 0.5	NS
Cl (mmol/L)	103.0 ± 4.5	101.5 ± 4.3	NS
CO_2 (mmol/L)	25.8 ± 2.8	25.9 ± 2.6	NS
Ca (mmol/L)	2.2 ± 0.2	2.2 ± 0	NS
P (mmol/L)	1.1 ± 0.2	1.1 ± 0.12	NS

ICIs have been shown to potentially have antiviral effects by decreasing T-cell exhaustion and enhancing virus-specific T-cell responses in HBV infections. Studies have demonstrated sustained antiviral effects of the PD-L1 blockade in chronic hepatitis B infections (Fisicaro et al., 2010). In a pilot study of virally suppressed HBeAg–patients, the checkpoint blockade was well tolerated and led to a decline in HBsAg levels in most patients (Gane et al., 2019). These findings support the results of our current study, which showed a decrease in HBsAg levels, particularly in patients with a baseline HBsAg level >1000 IU/mL. Our data suggest that ICIs may also have a role in antiviral functions (Hagiwara et al., 2022; Pan et al., 2022).

The incidence of immune-related adverse events in most solid tumors is reported to be between 17.1% and 27%, with 4%–6% of them being grade 3/4. However, in liver tumor patients, the incidence of irAEs is higher, ranging from 42.9% to 54.6% (Sangro et al., 2020; Sonpavde et al., 2021), with a grade 3/4 incidence of 10.7% (Julien et al., 2020). Our study, consistent with previous research studies, showed an incidence of irAEs of 38.46% and a grade 3/4 incidence of 13.33%. Skin events were the most common, followed by gastrointestinal and liver events. Among the irAEs observed in our patients, the top three were thyroid, liver, and skin-related events. We also noted a rare case of hyperlipidemia, which may be a potential irAE, but further clinical studies are necessary for its validation.

The most frequently reported gastrointestinal adverse reactions to ICI treatment include decreased appetite, nausea, vomiting, diarrhea, and constipation. In our study, the incidence of gastrointestinal adverse

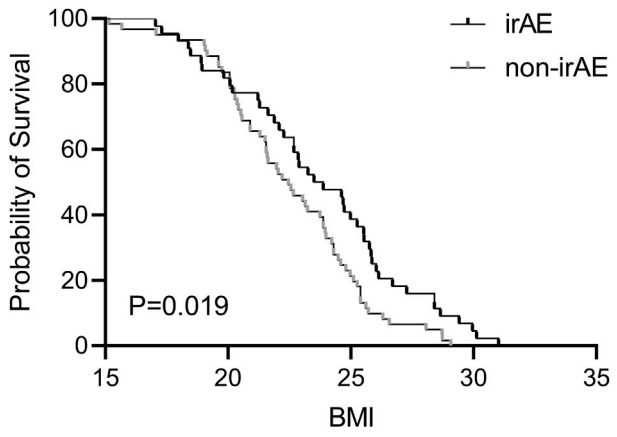

FIGURE 2
Association of irAEs with the risk factors of liver cancer patients and the Kaplan–Meier curve of BMI.

reactions did not rank among the top few adverse reactions. This could be due to the prophylactic use of antiemetic drugs, such as serotonin receptor antagonists and hormones, prior to ICI treatment.

Hepatic irAEs typically occur between 4 and 12 weeks after ICI treatment. Among patients with non-liver tumors, the incidence of hepatic irAEs is around 4%–6%, with 1%–2% of patients experiencing grade 3/4 adverse reactions (Julien et al., 2020). However, for patients with liver tumors, the incidence and severity of hepatic irAEs are important considerations. Previous studies have reported that hepatic irAEs occur in 4.3%–24.3% of patients with liver tumors, with grade 3/4 reactions occurring in 6.5%–6.9% of cases (Chen et al., 2020; Julien et al., 2020; He et al., 2021). In our study, only 13.21% of the patients developed full-grade hepatitis, with 9.43% experiencing grade 3/4 hepatitis. These results suggest that ICI therapy for liver tumors is generally safe.

The median time for the diagnosis of delayed irAEs was 6 months, following the treatment with ICIs, within a range of 3–28 months (Couey et al., 2019). For melanoma patients treated with anti-PD-1 therapy for more than 12 months, delayed irAEs occurred in 5.3% of cases (Julien et al., 2020). In a clinical trial for advanced HCC, 15.5% of patients experienced delayed irAEs, with grade 3/4 events being observed in 5.8% of patients after 100 days of follow-up (Julien et al., 2020). To date, there have been no reports of delayed irAEs related to liver tumors in the real world. In our study, the incidence of delayed irAEs was 0.94%, providing valuable reference for evaluating the occurrence of delayed irAEs in liver tumors in the real world.

Previous studies have shown that a higher BMI increases the risk of irAEs in patients with NSCLC, melanoma, renal cell carcinoma, urothelial carcinoma, and squamous cell carcinoma of the head and neck, suggesting that BMI may contribute to irAEs (Cortellini et al., 2020; Gülave et al., 2021; Leiter et al., 2021). In our study of patients with primary liver cancer, we observed that patients with irAEs had a higher BMI compared to non-irAE patients, with overweight patients experiencing an increased incidence of irAEs. These findings suggest that an elevated BMI may be a risk factor for irAEs, possibly due to the increased PD-1/PD-L1 expression in obesity-related immune cells (Ilavská et al., 2012). However, further prospective studies are needed to investigate this association in more detail.

Immunotherapy revolutionizes the therapeutic landscape of several tumor types, playing an important role in combination therapy of patients with advanced HCC. However, only a fraction of HCC patients benefit from immunotherapy (Pinato et al., 2020). Thus, identifying reliable response predictors would improve the efficacy of immunotherapy for HCC patients (Di Federico et al., 2022). PD-L1 and

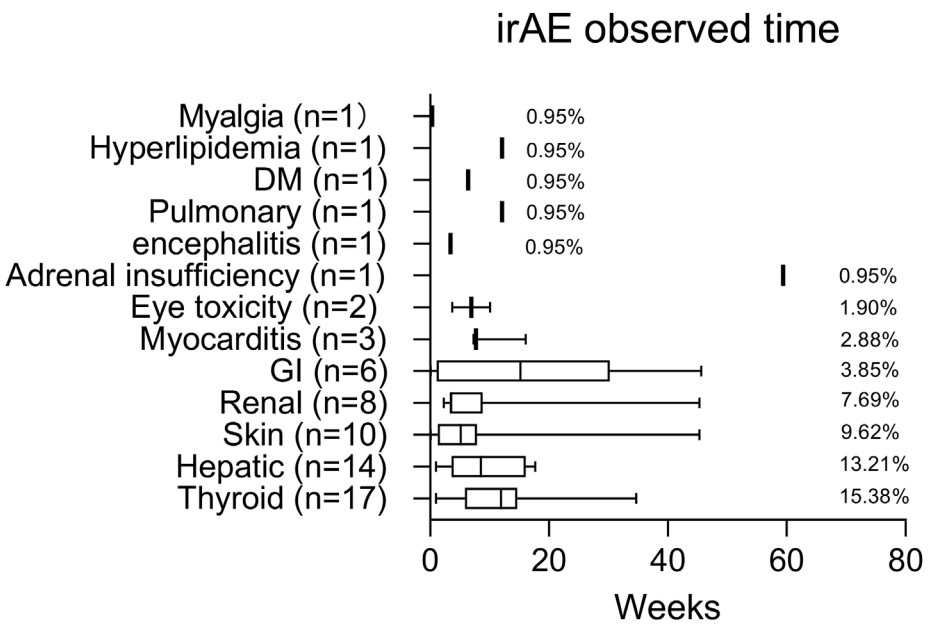

FIGURE 3
Median time (weeks) to irAE occurrence in PD-1-treated liver cancer patients.

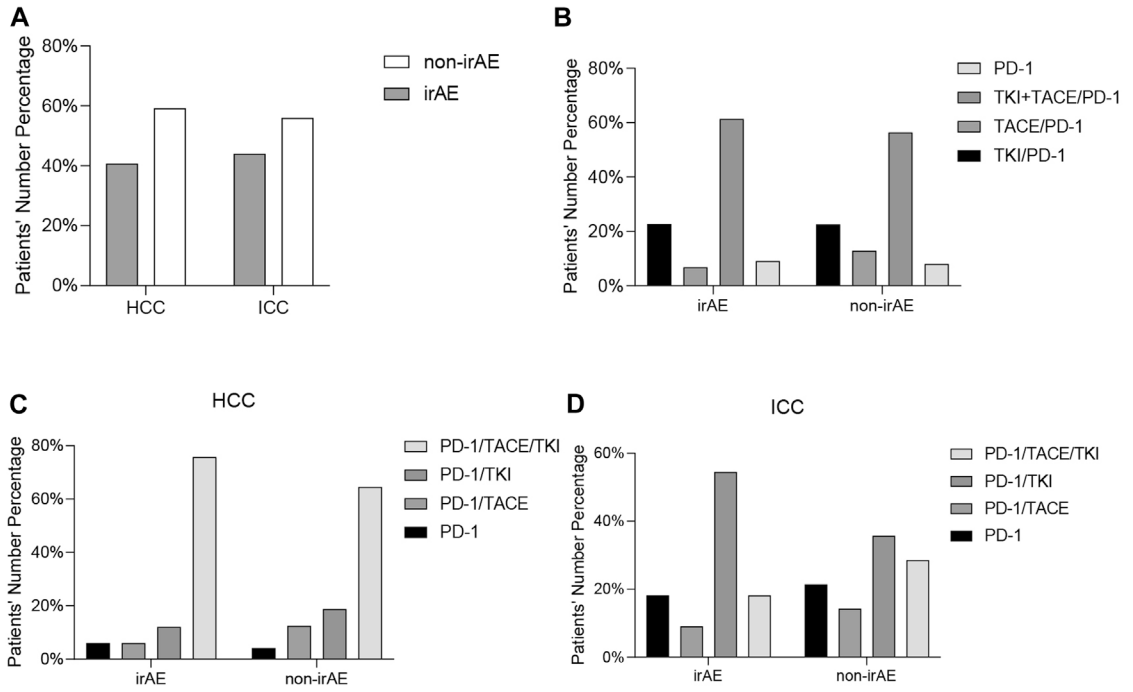

FIGURE 4
Comparison of the immune-related adverse events between HCC patients and ICC patients **(A)**. Comparison of immune-related adverse events between all patients **(B)**, HCC patients **(C)**, and ICC patients **(D)** receiving PD-1 inhibitors alone or in combination with TKI and TACE.

TABLE 3 Laboratory test with hepatic irAE patients.

Laboratory test	Baseline	irAE
ALT (IU/L)	53 ± 50	157 ± 282
AST (IU/L)	66 ± 61	140 ± 133
AKP (IU/L)	193 ± 110	285 ± 210
GGT (IU/L)	182 ± 146	261 ± 228
TBIL (μmol/L)	21 ± 13	50 ± 57
ALB (g/L)	34 ± 7	33 ± 8
TBA (μmol/L)	14 ± 11	20 ± 13

tumor mutational burden (TMB), gut microbiota, and several other potential predictors for HCC are currently being evaluated (Rizzo et al., 2021b; 2022). More recently, there have been several reports that cancer patients with 1/10 tumor cells expressed PD-L1 and PD-L1 have worse outcomes (shorter recurrence-free survival (RFS) and OS following immunotherapy) (Lei et al., 2020). A high TMB is associated with higher mutation rates and formation of neoantigens and enhanced anti-cancer immune responses, improving the clinical outcomes (Samstein et al., 2019; Rizzo et al., 2021a). However, data on the role of TMB and MSI as predictive biomarkers for HCC are scarce and limited to clinical trials or case reports (André et al., 2020; Merino et al., 2020). A study suggests that there are potential predictive roles of the MMR, MSI, TMB, and PD-L1 expression detection in ICC patients (Ricci et al., 2020).

Early crossing of survival curves in randomized clinical trials (RCTs) of immune checkpoint blockers suggests excess mortality in the first month of immunotherapy (Ricci et al., 2020). However, combined therapy substantially reduced early deaths compared to single immunotherapy (Viscardi et al., 2022).

Immunotherapy remains a significant challenge for patients with liver tumors. While it has been successfully applied to HCC patients with compensated cirrhosis or Child–Pugh A status, there is limited application in those with decompensated cirrhosis (Child–Pugh score B or C) or patients who have undergone liver transplantation (Akce et al., 2022). Although the application of atezolizumab/bevacizumab as the first-line treatment for advanced HCC has significantly improved clinical outcomes, there is a lack of understanding about the appropriate second-line treatment for these patients after immunotherapy (Wong et al., 2022).

Sipuleucel-T (a cancer vaccination) is currently approved for the treatment of metastatic castration-resistant prostate cancer with promising outcomes. Such a concept could also be utilized in HCC patients in the future, in combination with immunotherapy. Additionally, more attention may be focused on immune drug resistance, which is still the main contributing factor restricting the development of ICIs (Chen et al., 2022).

There are some limitations in the current study. First, this was a single-center retrospective study, which should be confirmed in the future as prospective multicenter studies. Second, the use of different PD-1 inhibitors may have impacted the uniformity of the treatment procedures. Third, the follow-up period was relatively short, and some patients were still receiving ICI treatment at the end of the study, which may

TABLE 4 Univariate analyses of irAEs' earliest occurrence time with Cox regression models.

Covariate	Univariate analysis (n = 44)			
	HR	95.0% CI		*p*-value
Group 1				
PD-1	Reference			
PD-1/TACE	0.099	0.019	0.513	0.006
PD-1/TKI	0.513	0.148	1.778	0.293
PD-1/TKI/TACE	0.169	0.051	0.565	0.004
Group 2				
TKI	Reference			
Non-TKI	2.410	1.124	5.165	0.095
Group 3				
TACE	Reference			
Non-TACE	0.273	0.132	0.566	<0.0001

Comparison of irAEs' earliest occurrence time between patients receiving PD-1 inhibitors alone or in combination with TKI and TACE.

compromise the statistics. The sample size was relatively small, and the HCC patients were the residents of the eastern coast of China. We will verify such information from the HCC patients from different regions and/or countries with different genetic backgrounds in the future.

Conclusion

This study provides important real-world evidence on the safety of combining immune checkpoint inhibitors (ICIs) with targeted drugs and interventional therapy and the risk of HBV reactivation in patients with HBV-related liver cancer. Effective antiviral prophylaxis is critical to ensuring the safety of ICI therapy in these patients. Our findings suggest that BMI may be a potential risk factor for irAEs, with overweight patients being more susceptible. While ICIs combined with targeted drugs or TACE therapy have a manageable safety profile, the occurrence of irAEs still requires close monitoring. Notably, the combined TACE treatment delayed the earliest occurrence of irAEs, and further investigation is warranted to understand the underlying mechanism.

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 Ethics Committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine. Written informed consent for participation was not required for this study in accordance with the national legislation and institutional requirements.

Author contributions

SL, ZC, and WC conceived the case and collected data. SL, XYW, and MF drafted the manuscript. LL, YD, and KL assisted with manuscript writing, editing, and detailed search of references. LQ provided support in data analysis and in manuscript writing. GZ, SB, and HW designed the experiment and revised the manuscript. All authors read and approved the final 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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1164309/full#supplementary-material

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Predictive model of chemotherapy-related toxicity in elderly Chinese cancer patients

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Purpose: Older cancer patients are more likely to develop and die from chemotherapy-related toxicity. However, evidence on drug safety and optimal effective doses is relatively limited in this group. The aim of this study was to develop a tool to identify elderly patients vulnerable to chemotherapy toxicity.

Patients and methods: Elderly cancer patients ≥60 years old who visited the oncology department of Peking Union Medical College Hospital between 2008 and 2012 were included. Each round of chemotherapy was regarded as a separate case. Clinical factors included age, gender, physical status, chemotherapy regimen and laboratory tests results were recorded. Severe (grade ≥3) chemotherapy-related toxicity of each case was captured according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 5.0. Univariate analysis was performed by chi-square statistics to determine which factors were significantly associated with severe chemotherapy toxicity. Logistic regression was used to build the predictive model. The prediction model was validated by calculating the area under the curve of receiver operating characteristic (ROC).

Results: A total of 253 patients and 1,770 cases were included. The average age of the patients was 68.9 years. The incidence of grade 3–5 adverse events was 24.17%. Cancer type (non-GI cancers), BMI<20 kg/m^2, KPS<90%, severe comorbidity, polychemotherapy, standard dose chemotherapy, low white blood cells count, anemia, low platelet cells count, low creatine level and hypoalbuminemia were associated with severe chemotherapy-related toxicity. We used these factors to construct a chemotherapy toxicity prediction model and the area under the ROC curve was 0.723 (95% CI, 0.687–0.759). Risk of toxicity increased with higher risk score (11.98% low, 31.51% medium, 70.83% high risk; $p < 0.001$).

Conclusion: We constructed a predictive model of chemotherapy toxicity in elderly cancer patients based on a Chinese population. The model can be used to guide clinicians to identify vulnerable population and adjust treatment regimens accordingly.

KEYWORDS

chemotherapy, gastrointestinal cancers, drug-related adverse effects, elderly, cancer

Introduction

Cancers are age-related diseases (Chang et al., 2019). According to the data on GLOBOCAN, approximately 50% of new diagnosed cancer patients in 2020 are elderly people over 65 years old (Sung et al., 2021). However, there are still many knowledge gaps in the treatment of elderly cancer patients (Hurria et al., 2014).

While older patients may respond similarly to anticancer treatments as younger patients, treatment-related toxicity remains a concern (Macchini et al., 2019). Studies have found that older patients are more likely to experience chemotherapy-related adverse events (Trumper et al., 2006; Muss et al., 2007; Asmis et al., 2008). Poor tolerability in the elderly population may be due to many factors, including age-related deterioration of multiple organ functions, comorbidities, polypharmacy, and other problems that can lead to altered pharmacokinetics and pharmacodynamics of chemotherapy drugs (Brunello et al., 2009; Feliu et al., 2018).

Dose reduction as a strategy to improve patient tolerability while preserving the antitumor effect has been identified as a promising approach (Hall et al., 2021). However, the elderly population is a highly heterogeneous group, with chronological age often not reflecting functional status and chemotherapy tolerance (Hernandez Torres and Hsu, 2017). Moreover, current guidelines for cancer treatment primarily rely on evidence obtained from clinical trials, which often exclude older patient population (Joharatnam-Hogan et al., 2020). Therefore, more evidence is needed to identify elderly populations at risk and guide adjustments of antitumor drug doses.

Several predictive tools have been developed to assess the risk of chemotherapy toxicity, including the Cancer and Aging Research Group (CARG) score and the Chemotherapy Risk Assessment Scale for High-Age Patients (CRASH) Score (Hurria et al., 2011; Extermann et al., 2012). However, it should be noted that these models were primarily based on data from the Caucasian population and have limited applicability to Asian populations.

In this article, we collected data of elderly cancers patients from a tertiary hospital in inland China and analyzed the incidence of severe chemotherapy-related adverse events. We aimed at predicting the risk of chemotherapy toxicity using a logistic regression model and this predictive model should give more suggestion when discussing the risks and benefits of chemotherapy with older adults.

Methods

Setting and patient

This study retrospectively analyzed elderly cancer patients who attended Peking Union Medical College Hospital between 2008 and 2012. Patient data were extracted, encrypted, and de-identified by 2 professional researchers in 2013. The Ethics Committee at Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College approved the study and waived the need for patient consent because of the retrospective design of this study.

1,453 cancer patients were reviewed, and 253 patients were enrolled. The inclusion criteria were patients (1) who were older than or equal to 60 years; (2) with a clear pathological diagnosis of malignancy or lymphoma; and (3) receiving at least one chemotherapy treatment. Patients whose diagnosis was unclear or who did not receive chemotherapy were excluded.

Each chemotherapy cycle received by each patient is considered as a separate case in our study. In total, 1,770 cases are included.

Primary outcome

The primary endpoint was the occurrence of severe hematologic and non-hematologic chemotherapy-related toxicity (grade 3 [hospitalization indicated], grade 4 [life threatening], and grade 5 [treatment-related death]), graded using National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v5.0 criteria (National Cancer Institute, 2017). This endpoint was chosen because most guidelines recommend dose adjustment when severe toxicity (grade≥ 3) occurs.

Data collection

Patient demographic data (gender and age), tumor-related conditions (type and stage), body mass index (BMI), and comorbidities were collected. The Charlson comorbidity index (CCI) score was used to assess the severity of comorbidity (Charlson et al., 1987). In calculating the CCI score, metastatic solid tumor was excluded as comorbid conditions, given that our data itself is a cohort of tumor patients and most of them had distant metastases.

Before each chemotherapy cycle, the following data were captured: (1) Chemotherapy regimen, number of chemotherapy drugs, and chemotherapy doses. (2) Eastern Cooperative Oncology Group (ECOG) Performance Status Scale and Karnofsky score (KPS). (3) Laboratory variables included but are not limited to white blood cells, hemoglobin, platelets, transaminases, creatinine, and albumin.

Statistical analyses

We performed descriptive statistics of chemotherapy-related adverse events in all patients and calculated the incidence of hematological and non-hematological toxicities.

Model development

First, we used chi-square (χ^2) tests to identify variables associated with grade 3–5 chemotherapy-related adverse events. Variables included age, gender, tumor type (non-gastrointestinal cancers or gastrointestinal cancers), stage (≤3 or 4), number of chemotherapy drugs (single or multiple drugs), chemotherapy doses (reduced or standard doses), BMI (<20 kg/m^2 or ≥20 kg/m^2), ECOG (>1 or ≤1), KPS (<90% or ≥90%), comorbidity score (CCI≥4 or <4) and multiple laboratory variables.

Variables with p-values less than 0.1 and certain clinical variables strongly associated with the outcome would be selected as model factors. We established the predictive model by a multivariate logistic regression model. The Youden Index

TABLE 1 Patients characteristics.

Characteristics	No. of patients	% Patients
Baseline Characteristics (N = 253)		
Gender		
Male	168	66.40
Female	85	33.60
Age, years (Average: 68.9)		
60–64	63	24.90
65–69	75	29.65
70–74	58	22.92
75–79	43	17.00
≥80	14	5.53
Cancer type		
GI	172	67.98
Esophageal Cancer	7	4.07
Gastric Cancer	34	19.77
Colorectal Cancer	78	45.35
Pancreatic Cancer	17	9.88
Bile Duct Cancer	1	0.58
Liver Cancer	2	1.16
Non-GI	80	31.62
Genitourinary Cancer	8	10.00
Lung Cancer	56	70.00
Lymphoma	8	10.00
Melanoma	3	3.75
Miss	1	0.40
Cancer stage		
0-III	113	44.66
IV	110	43.48
Miss	30	11.86
Chemotherapy Cycle		
1–3	46	18.18
4–6	72	28.46
7+	135	53.36
Comorbidity		
None or less severe	193	76.28
Severe	60	23.72
Available Characteristics of Cases		
KPS (%) (n = 1,429)		
≥90	1,103	77.19
< 90	326	22.81
ECOG (n = 1,470)		
≤1	1,336	90.88
>1	134	9.12
BMI, kg/m^2 (n = 1,490)		
<18	151	10.13
[18, 24)	865	58.05
[24, 28)	372	24.97
≥28	102	6.85
Numebr of chemotherapy agents (n = 1,757)		
1	336	19.12
≥2	1,421	80.88
Dose (n = 1,489)		
Reduced	751	50.44
Standard	738	49.56

Note: GI, gastrointestinal cancer.

(Youden, 1950) was used to identify the cut point with the highest sensitivity and specificity when classifying the presence or absence of toxicity. The discrimination of models was evaluated by calculating the area under the receiver operating characteristic (ROC) curve.

Developing the scoring system

A risk score for each risk factor was calculated by dividing the coefficient of the variable by the lowest coefficient in the model, rounded to the nearest 0.5 times. (Concato et al., 1993; Walter et al., 2001). After that, the sum of scores for each chemotherapy case was calculated. The sample was divided into three risk strata (low, medium, and high risk) based on approximate quartiles of risk score with the middle two quartiles combined. The difference in toxicity incidence among the strata was evaluated by χ^2 test.

Model validation

The model was internally validated. We obtain the area under the ROC curve (AUC) of the model. If the AUC is larger than 0.7, it means the model is valid. All statistical analyses were performed by using SPSS.

Results

Characteristics of patients and cases

The basic information of the patients was listed in Table1. Male patients accounted for 66.4% and female patients accounted for 33.6%. The average age of patients was 68.9 years old. More than 75% of patients were older than 65 years old, but the proportion of the oldest old patients (≥80 years old) was relatively small, accounting for only about 5%. Staging IV account for 43.48%.

TABLE 2 Treatment-related adverse events.

	Cases		Severe toxicity	
	No.	%	No.	%
Non-hematologic				
Weakness	257	14.52	6	2.33
Weight loss	80	4.52	12	5.00
Rash	79	4.46	3	3.80
Alopecia	31	1.75	0	0
Fever	93	5.25	4	4.3
Infection	21	1.19	6	28.57
Muscle Pain	48	2.71	0	0
Headache and Dizziness	56	3.16	0	0
Insomnia	46	2.60	0	0
Cough	30	1.69	0	0
Dyspnea	47	2.66	1	2.13
Nausea	398	22.49	8	2.01
Vomiting	200	11.30	7	3.50
Lack of Appetite	376	21.24	7	1.86
Diarrhea	186	10.51	12	6.45
Constipation	171	9.66	0	0
Abdominal Pain and Bloating	127	7.18	0	0
Other Gastrointestinal Disorders	57	3.22	0	0
Neurotoxicity	129	7.29	1	0.78
Edema	59	3.33	0	0
Thromboembolic Event	22	1.24	0	0
ALT Elevation (N = 654)	66	10.09	3	4.55
Abnormal Total Bilirubin (N = 637)	89	13.97	0	0
Creatinine Increased (N = 650)	42	6.46	0	0
Hypokalemia (N = 583)	61	10.46	0	0
Hypoalbuminemia (N = 559)	120	21.47	0	0
Hematologic				
White blood cell count decreased (N = 818)	578	70.66	118	20.42
Anemia (N = 900)	576	64.00	46	7.99
Neutrophil count decreased (N = 918)	421	45.86	150	35.63
Platelet count decreased (N = 909)	306	33.66	55	17.97
Total				
Total Adverse Events (N = 1770)	1,411	79.72	341	24.17

Note: Severe toxicity refers to grade 3–5 toxicity, defined by National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v5.0 criteria.

The most common cancer type was gastrointestinal cancer (172 cases, about 67.98% of 253 patients). Among GI cancer, gastric cancer accounted for about 20% (34 cases) and colorectal cancer accounted for about 45% (78 cases). Among non-GI tumors, lung cancer accounted for the largest proportion (56 cases, 70%). As for patients' treatments, 18.18% of patients experienced 1–3 rounds of chemotherapy, 28.46% experienced 4-6 rounds, and 53.36% experienced more than 7 rounds of chemotherapy. 76.28% of patients had none or less severe comorbidity (CCI <4), whereas 23.72% of patients had severe comorbidity (CCI≥ 4). The most

common comorbidities were cardiovascular disease, diabetes mellitus and chronic respiratory disease, consistent with previous surveys of chronic disease burden in the elderly population (Supplementary Figure S1) (Prince et al., 2015).

Most patients had relatively good physical status during each chemotherapy cycle: Among the 1,429 cases, which KPS scores were available, 77.19% of patients (1,103 cases) had a KPS score≥ 90%. Among the 1,470 cases, which ECOG scores were available, 90.88% (1,336 cases) had an ECOG score ≤1. More than half of the cases (58.05%, 865 cases from total 1,490 valid BMI records) had normal weight, 31.82% (474 cases) were overweight or obese (BMI >24 and 28 kg/m^2, based on the new Chinese criteria (Pan et al., 2021)) and 10.13% (151 cases) were underweight (BMI <18 kg/m^2). Besides, in 1757 total valid data of the number of chemotherapy agents, approximately 80% of cases were treated with a multidrug chemotherapy regimen and about 20% were treated with single-agent chemotherapy. In detail, 56.55% of the regimen contained fluoropyrimidine (such as capecitabine, fluorouracil and tegafur), 60.28% contained platinum, and about 20% contained taxanes (Supplementary Table S1). In addition, in 1,489 total valid data of chemotherapy dose, 50.44% of the patients received physician-determined reduced-dose chemotherapy, while the others used the guideline-recommended standard dose. Of patients who underwent dose adjustment, more than half of the patients (64.85%) received chemotherapy with a reduction of 20%–35%, 29.69% with a reduction of 35%–50%, and only 5.46% with a reduction of more than 50% (Supplementary Table S2).

Chemotherapy-related toxicity

Consistent with previous studies (Trumper et al., 2006; Muss et al., 2007; Asmis et al., 2008), the incidence of chemotherapy-related toxicity in elderly tumor patients was high, with 79.72% of patients experiencing any grade of adverse events, of which about 24.16% were grade 3–4 adverse events (Table 2). 12 patients died but were considered not to be directly related to chemotherapy toxicity.

Among all non-hematologic adverse events in total 1770 cases, nausea (398 [22.49%]), lack of appetite (376 [21.24%]), hypoalbuminemia (120 [21.47%]) and weakness (257 [14.52%]) were most common, but mostly to a lesser extent. Although a low proportion of the overall cases, infection was the most common severe non-hematologic adverse events: In 1770 cases, only 21 cases had infection but 6 of them (1.19%) were grade 3–5 toxicity, which was the highest proportion of grade 3–5 toxicity among all non-hematologic adverse events, accounting for 28.57%.

The incidence of hematological toxicity was higher than non-hematological toxicity. Among valid data: The most common hematologic adverse events were white blood cell decreased (578 [70.66%]), followed by anemia (576 [64%]) and thrombocytopenia (306 [33.66%]). Among severe hematologic adverse events, neutropenia was the most common (150 [35.63%]).

Chemotherapy-related toxicity predict model

We assessed the association between severe chemotherapy-related toxicity (Grade≥ 3) and multiple clinical variables (Table 3). There are 12 variables significantly associated with severe chemotherapy-related toxicity: cancer type (non-GI, $p < 0.001$), number of chemotherapy agents (polychemotherapy, $p = 0.042$), chemotherapy dose (standard dose, $p = 0.078$), BMI (<20 kg/m^2, $p < 0.001$), KPS (<90%, $p < 0.001$), ECOG (>1, $p = 0.036$), comorbidity (CCI≥4, $p = 0.002$), low white blood cell (<4 × 10^9/L, $p < 0.001$), low neutrophils (<2×10^9/L, $p < 0.001$), anemia (hemoglobin<110 g/L, $p < 0.001$), low platelets (<100×10^9/L, $p < 0.001$), hypoalbuminemia (albumin<35 g/L, $p < 0.001$), and low creatine level (<59 μmol/L, $p < 0.001$). Outliers in these factors can significantly increase the probability of severe chemotherapy-related toxicity. Focusing on BMI, for example, of the 1,176 non-severe toxicity cases, the proportion of BMI<20 kg/m^2 is 20.07% (236 cases), which is increasing significantly to 28.66% (90 cases) in 314 cases with severe toxicity. At the same time, the chi-square (χ^2) test obtained $p < 0.001$, which showed that lower BMI was significantly positively correlated with the occurrence of severe chemotherapy-related toxicity.

We selected 11 variables to construct a chemotherapy-related toxicity predictive model (Table 4). The variables included cancer type (non-GI cancer), BMI< 20 kg/m^2, KPS< 90%, severe comorbidity, polychemotherapy, standard dose chemotherapy and 5 laboratory variables (low white blood cells count, anemia, low platelet cells count, low creatine level and hypoalbuminemia.) Each variable was assigned a different risk score (ranged 1–3), with a total score of 21.

Model validation

Risk score ranges from 0 to 21 points and was divided into three groups (low-risk group, 0 to 6 points; medium-risk group, 6.5 to 12 points; high-risk group, 12.5 to 21 points). Most patients (57.54%) were classified as low-risk group, 40.26% of patients were classified as medium-risk group, and 2.2% were classified as high-risk group. The risk of toxicity increased with increasing risk score (11.98% in the low-risk group, 31.51% in the medium-risk group, and 70.83% in the high-risk group; $p < 0.001$; Table 5). We examined the internal validation of this model: The area under the ROC curve for the predictive model is 0.723 ([95% CI, 0.687 to 0.759]. Figure 1), suggesting good predictive power of severe chemotherapy toxicity.

Discussion

The tolerance of chemotherapy in elderly cancer patients is a concern. Predicting the risk of chemotherapy toxicity in advance can help clinicians identify vulnerable populations.

Several models have been developed to predict chemotherapy toxicity. In 2011, Hurria et al. constructed the CARG score, which is a predictive tool and a risk stratification schema that aims to identify older adults at low, intermediate, or high risk of chemotherapy toxicity (Hurria et al., 2011). The variables included in the model are age, tumor type, treatment intensity, laboratory test values, and a 5-question brief geriatric assessment. The CARG tool is simple to use and has been validated in many studies (Kotzerke et al., 2019; Zhang et al., 2019; Ostwal et al., 2021). In the same year, Extermann et al.

TABLE 3 Association between case characteristics and toxicity.

Variable	Cases		Non-severe toxicity		Severe toxicity		*p*-Value
	No.	%	No.	%	No.	%	
Demographics							
Gender							
	1,770		1,417	80.06	353	19.94	0.118
Male	1,146	64.75	930	65.63	216	61.19	
Female	624	35.25	487	34.37	137	38.81	
Age, years							
	1,770		1,417	80.06	353	19.94	0.136
< 69	920	51.98	724	51.09	196	55.52	
≥69	850	48.02	693	48.91	157	44.48	
Tumor and treatment							
Cancer type							
	1,768		1,415	80.03	353	19.97	<0.001
GI	1,343	75.96	1,126	79.58	217	61.47	
non-GI	425	24.04	289	20.42	136	38.53	
Number of chemotherapy agents							
	1,757		1,410	80.25	347	19.75	0.042
1	336	19.12	283	20.07	53	15.27	
≥2	1,421	80.88	1,127	79.92	294	84.73	
Dose							
	1,489		1,174	78.84	315	21.16	0.078
Reduced	751	50.44	606	51.62	145	46.03	
Standard	738	49.56	568	48.38	170	53.97	
Cancer stage							
	1,607		1,309	81.46	298	18.54	0.834
I ~ III	730	45.43	593	45.30	137	45.97	
IV	877	54.57	716	54.70	161	54.03	
Geriatric assessment							
KPS, %							
	1,429		1,120	78.38	309	21.62	<0.001
≥90	1,103	77.19	896	80	207	67	
<90	326	22.81	224	20	102	33	
BMI, kg/m^2							
	1,490		1,176	78.93	314	21.07	<0.001
≥20	1,164	78.12	940	79.93	224	71.34	
<20	326	21.88	236	20.07	90	28.66	

(Continued on following page)

TABLE 3 (*Continued*) Association between case characteristics and toxicity.

Variable	Cases		Non-severe toxicity		Severe toxicity		*p*-Value
	No.	%	No.	%	No.	%	
Comorbidity							
	1,770		1,417	80.06	353	19.94	0.002
None or less severe	1,353	76.44	1,105	77.98	248	70.25	
severe	417	23.56	312	22.02	105	29.75	
ECOG							
	1,470		1,167	79.39	303	20.61	0.036
≤1	1,336	90.88	1,070	91.69	266	87.79	
>1	134	9.12	97	8.31	37	12.21	
Laboratory variables							
White blood cell, × 10^9/L							
	1,580		1,261	79.81	319	20.19	<0.001
≥4	1,160	73.42	963	76.37	197	61.76	
<4	420	26.58	298	23.63	122	38.24	
Hemoglobin, g/L							
	1,579		1,260	79.80	319	20.20	<0.001
≥110	1,103	69.85	928	73.65	175	54.86	
<110	476	30.15	332	26.35	144	45.14	
Neutrophils, × 10^9/L							
	1,559		1,247	79.99	312	20.01	<0.001
≥2	1,176	75.43	971	77.87	205	65.71	
<2	383	24.57	276	22.13	107	34.29	
Platelets, × 10^9/L							
	1,574		1,256	79.80	318	20.20	<0.001
≥100	1,362	86.53	1,115	88.77	247	77.67	
<100	212	13.47	141	11.23	71	22.33	
Creatine level, μmol/L							
	1,500		1,195	79.67	305	20.33	<0.001
≥59	1,192	79.47	974	81.51	218	71.48	
<59	308	20.53	221	18.49	87	28.52	
Albumin, g/L							
	1,300		1,034	79.54	266	20.46	<0.001
≥35	1,127	86.69	915	88.49	212	79.70	
<35	173	13.31	119	11.51	54	20.30	
Creatine clearance, Cockcroft-Gault, mL/min							
	1,382		1,087	78.65	295	21.35	0.438
≥60	1,078	78	843	77.55	235	79.66	
<60	304	22	244	22.45	60	20.34	

(Continued on following page)

TABLE 3 (*Continued*) Association between case characteristics and toxicity.

Variable	Cases		Non-severe toxicity		Severe toxicity		*p*-Value
	No.	%	No.	%	No.	%	
ALT, U/L							
	1,500		1,195	79.67	305	20.33	0.92
≤40	1,423	94.87	1,134	94.90	289	94.75	
>40	77	5.13	61	5.10	16	5.25	

TABLE 4 Chemotherapy-related toxicity predictive model.

Toxicity type	Prevalent Cases (N = 1,088)		Severe Toxicity (N = 230)		Score
	No.	%	No.	%	
non-GI cancer	289	26.56	91	31.49	2.5
BMI<20 kg/m^2	237	21.78	68	28.69	2
KPS<90%	250	22.98	74	29.60	2
Severe comorbidity	257	23.62	66	25.68	1.5
Polychemotherapy	928	85.29	198	21.34	1.5
Standard dose chemotherapy	553	50.83	112	20.25	1
WBC<4×10^9/L	299	27.48	98	32.78	2.5
Hemoglobin<110 g/L	340	31.25	105	30.88	2
PLT <100×10^9/L	141	12.96	57	40.43	3
Serum creatine <59 μmol/L	222	20.40	69	31.08	1.5
Albumin <35 g/L	149	13.69	47	31.54	1.5
Total score					21

TABLE 5 Ability of risk score to predict chemotherapy toxicity.

Risk strata	Total case		Non-severe toxicity		Severe toxicity		*p*-Value	AUC
	No.	%	No.	%	No.	%		
By total score	1,088	100	858	78.86	230	21.14	<0.001	0.723
0–6 (low)	626	57.54	551	88.02	75	11.98		
6.5–12 (mid)	438	40.26	300	68.49	138	31.51		
12.5–21 (high)	24	2.20	7	29.17	17	70.83		

published the CRASH Score, which is more comprehensive but relatively complex to use. Among Asian populations (Extermann et al., 2012), the Korean Cancer Study Group (KCSG) score have been developed by Kim and his colleagues but has not yet been widely used (Kim et al., 2018).

In this retrospective single-center study, we sought to develop an objective predictive model based on a Chinese population cohort. In our model, we included tumor types (non-GI cancers), BMI, KPS, severe comorbidity, chemotherapy regimens (multidrug and standard dose chemotherapy) and 5 laboratory variables to predict the risk of chemotherapy-related toxicity.

The effect of BMI, KPS or ECOG score, comorbidity and chemotherapy regimens on chemotherapy tolerance have been widely discussed ((Hurria et al., 2011; Lee et al., 2011; Extermann et al., 2012; Dotan et al., 2020).

Among the laboratory variables, anemia is frequently diagnosed in elderly people and is associated with reduced overall survival. (Knight et al., 2004; Stauder et al., 2018). Anemia leads to increased serum free concentrations of many chemotherapeutic agents that need to bind to red blood cells, thereby increasing toxicity. (Schrijvers et al., 1999; Feliu et al., 2018). Similarly, hypoalbuminemia, an indicator of malnutrition, increases the

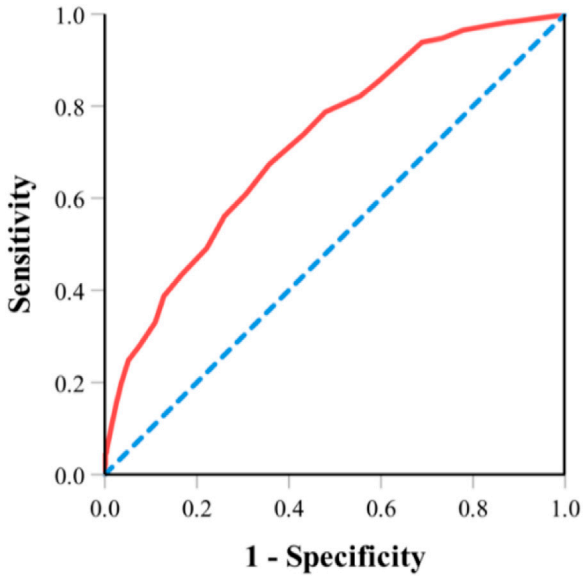

FIGURE 1
ROC curve for the predictive model. Note: Area under the ROC curve for the predictive model is 0.723 ([95% CI, 0.687 to 0.759]. ROC, receiver operating characteristic.

serum concentration of some drugs and leads to increased chemotherapy toxicity. (Schrijvers et al., 1999; Feliu et al., 2018).

The association between malnutrition and chemotherapy tolerance has been demonstrated in many studies. (Arrieta et al., 2010; Barret et al., 2011; Bozzetti, 2017). Low serum creatinine is a marker of reduced muscle mass associated with malnutrition, aging, and chronic disease, but is often overlooked in clinical practice (Cartin-Ceba et al., 2007; de Jong et al., 2022). In our cohort, there is a large proportion of patients (308/1,500) had a lower-than-normal serum creatinine level which is associated with high risk of chemotherapy-related toxicity. We also analyzed the effect of elevated serum creatinine level on the risk of toxicity but did not obtain statistically significant results, probably because of the small number of this group of people.

Interestingly, non-GI cancer type is associated with severe toxicity in our cohort, which is the opposite of the CARG study results. In the CARG study, patients with gastrointestinal (GI) or genitourinary (GU) tumors had a higher risk of chemotherapy toxicity (Hurria et al., 2011). We thought this may be related to the different toxicity profiles between different human races. Fluoropyrimidine drugs such as 5-fluorouracil, S-1 and capecitabine are recommended by many guidelines and frequently used in the treatment of GI cancers. (Park and Chun, 2013). Studies have found that fluoropyrimidine drugs cause a higher incidence of severe gastrointestinal toxicity in Caucasians than in Asians, partly because of polymorphic differences in the CYP2A6 gene. (Ajani et al., 2005; Haller et al., 2008; Chuah et al., 2011; Ma et al., 2012).

Notably, in our data, the risk of chemotherapy toxicity was not increased with age. This may be because half of our patients had already received dose-reduced regimen. In clinical practice, oncologists often reduce the doses for elderly and frail patients based on their clinical assessment to prevent severe toxicity. (Gajra et al., 2015). However, it is currently unknown whether the empirical adjustment of drug doses will result in optimal clinical outcomes. This uncertainty prompted the development of this predictive model to assess the risk of chemotherapy toxicity in older patients.

There are some limitations to this study. First, this study was a retrospective single-center analysis and more external validation is needed. A prospective clinical study is underway at our center, and further data is expected to confirm the utility of this predictive model.

Second, we did not include comprehensive geriatric assessments such as functional capabilities, cognitive status, emotional status, and social support. Although the ECOG score, comorbidity score, and laboratory variables can partially reflect the health status of elderly patients, they cannot replace the comprehensive geriatric assessment (CGA). However, the comprehensive geriatric assessment is still rarely used in China. In a recent study, the use of CGA tools was found to be only 56.9% in tertiary hospitals in China. (Wu et al., 2022). We are actively collaborating with geriatricians to introduce geriatric assessments into our center and plan to incorporate additional CGA components in future iterations of this model.

This study has some future directions. Many novel anti-tumor therapies such as targeted therapy, immunotherapy, and CAR-T therapy have emerged. However, there are less evidence on the use of these drugs in elderly patients. Toxicity prediction models should also be constructed and validated for these treatments. In addition, even though we successfully stratified patients according to their risk of chemotherapy toxicity, it is still unclear what percent of dose reduction should be applied to each group of patients.

In conclusion, we constructed a simple and objective model with 11 variables to predict chemotherapy-related toxicity in elderly cancer patients. This model aims to help clinicians identify vulnerable populations as well as formulate the best treatment and nursing strategies for elderly cancer patients.

Data availability statement

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

Author contributions

MG conceived and designed the study. YPZ, YH, and FL collected the data. YLZ performed the statistical analysis. YH and YLZ wrote the draft of the manuscript. 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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1158421/full#supplementary-material

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Management and prediction of immune-related adverse events for PD1/PDL-1 immunotherapy in colorectal cancer

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Programmed cell death protein (PD-1) is an important immunosuppressive molecule, which can inhibit interaction between PD-1 and its ligand PD-L1, further enhancing the T cell response and anti-tumor activity, which is called immune checkpoint blockade. Immunotherapy, represented by immune checkpoint inhibitors, has opened up a new era of tumor treatment and is gradually being applied to colorectal cancer recently. Immunotherapy was reported could achieve a high objective response rate (ORR) for colorectal cancer with high microsatellite instability (MSI), thus opening up a new era of colorectal cancer immunotherapy. Along with the increasing use of PD1 drugs in colorectal cancer, we should pay more attention to the adverse effects of these immune drugs while seeing the hope. Immune-related adverse events (irAEs) caused by immune activation and immune homeostasis during anti-PD-1/PD-L1 therapy can affect multi-organ and even be fatal in serious cases. Therefore, understanding irAEs is essential for their early detection and appropriate management. In this article, we review the irAEs that occur during the treatment of colorectal cancer patients with PD-1/PD-L1 drugs, analyze the current controversies and challenges, and point out future directions that should be explored, including exploring efficacy predictive markers and optimizing the paradigm of individualized immunotherapy.

KEYWORDS

colorectal cancer, immunotherapy, PD-1/PD-L1 inhibitors, immune-related adverse events, prediction

1 Introduction

Colorectal cancer is the third most common malignancy worldwide (Zheng et al., 2022). The latest global statistics show that in 2020 there were 1,148,515 new cases of colon cancer and 732,210 new cases of rectal cancer (Wei et al., 2020; Zheng et al., 2022). For decades, surgery, radiotherapy, and chemotherapy have been the main weapons used by physicians to fight colorectal cancer. However, there are problems with current treatment, especially for some patients who are not candidates for surgery in the advanced stage. In recent years, immunotherapy has emerged and the advent of immune checkpoint inhibitors (ICI) has opened up a new era in oncology treatment.

In recent years, many findings have confirmed that immunosuppressive molecules such as cytotoxic T lymphocyte-associated antigen 4 (CTLA4), PD-1and its ligand PD-L1 are seen

to be significantly overexpressed in the immune microenvironment of tumor patients (Mellman et al., 2011). PD-1 is an important immunosuppressive molecule. It regulates the immune system and promotes self-tolerance by down-regulating the immune system response to human cells, as well as by suppressing T-cell inflammatory activity. Significant upregulation of expression is seen in certain tumors, where PD-1 binding to its receptor PD-L1 initiates programmed death of T cells, allowing tumor cells to acquire immune escape (Postow et al., 2018; Seidel et al., 2018). By inhibiting the interaction between PD-1 and PD-L1, T-cell responses are enhanced and thus anti-tumor activity is increased, i.e., immune checkpoint blockade (Larkin et al., 2015). Checkpoint inhibitors targeting the PD-1 pathway are now approved for the treatment of a variety of tumors (Larkin et al., 2015; Motzer et al., 2015).

In 2015, Dung T.'s team first used pd1 drugs for the treatment of patients with dMMR/MSI-H metastatic colorectal cancer, and 10 previously treated patients were treated with the PD-1 inhibitor pembrolizumab, and the results showed an ORR of 40%, indicating that this group of patients may benefit from this treatment (Le et al., 2015). Since then, we have seen a new hope for the treatment of colorectal cancer, and subsequently, more teams have conducted related studies, all of which resulted in good therapeutic outcomes, further demonstrating the efficacy of PD-1 drugs in mCRC patients with dMMR (Overman et al., 2017; André et al., 2020; Stein et al., 2021; Haag et al., 2022). Besides, immunologic drugs in CRC may have good efficacy in patients with locally advanced rectal cancer (LARC). Currently, the first-line treatment for patients with LARC is still surgery combined with radiotherapy, which involves R0 survival and anal preservation. The VOLTAGE study investigated neoadjuvant immunotherapy with Nivolumab after long-course simultaneous radiotherapy for locally advanced rectal cancer, and the pathologic complete response (pCR) rate in the dMMR group reached 60% (Bando et al., 2022). Similar results have been obtained from some other studies in China and abroad that patients with locally progressive colorectal cancer receiving neoadjuvant immunotherapy can achieve a high pCR rate (Shamseddine et al., 2020; Lin et al., 2021; Hu et al., 2022). It can be seen that immunotherapy will undoubtedly play a great power in the future for both mCRC patients and LARC patients, and the application of PD-1 drugs in colorectal cancer is incomparably bright.

Along with the increasing use of PD-1 drugs in colorectal cancer, we must be concerned about the adverse effects of this class of immune drugs while seeing hope. By unbalancing the immune system, immune checkpoint blockade favors the development of autoimmune manifestations, also known as irAEs. Most of these adverse events can be managed by steroids to counteract lymphocyte activation. However, although steroid use causes irAEs to subside, the associated immunosuppression may impair the antitumor response (Kuehn et al., 2014; Darnell et al., 2020). It has been reported in the literature that severe irAEs not only do not benefit patients but may lead to death. In addition, the onset of irAEs is difficult to predict and can occur even after treatment is discontinued and persist for a long time. The expected frequency of AEs in immunotherapy, chemotherapy, and other treatment modalities differs due to the unique mechanism of action of ICIs. Therefore, understanding irAEs is crucial for their early detection and appropriate management and is more likely to further guide the use of PD-1 drugs in the field of colorectal cancer.

We conducted a systematic literature search of the pubMed, MEDLINE, Cochrane Library, EMBASE, China National Knowledge Infrastructure (CNKI), Wanfang Database, VIP Medical Information System, and China Biomedical Database (CBM) from inception to 15 December 2022. The search terms were composed of the following medical themes (MeSH) and additional conditions: (colorectal cancer/colorectal neoplasms/colorectal tumor) AND (programmed cell death protein/PD-1/PD-L1) AND (immune-related adverse events/irAEs). Furthermore, manual studies would be conducted to find potential references. Language was not an obstacle to publication.

2 Clinical application and management of irAEs in CRC patients treated with ICI

The 2021 version of the NCCN Guidelines changes the previous recommendations for detecting MMR/MSI status. The guidelines recommend universal MMR or MSI testing for all patients with a personal history of colon or rectal cancer. In addition to serving as a predictive marker for immunotherapy in advanced CRC settings, MSI/MMR status can also help identify individuals with Lynch syndrome and inform adjuvant treatment decisions in patients with stage II CRC. Firstly, we summarized the different applications of PD-1/PD-L1 inhibitors in a clinical study of CRC patients (Table 1).

2.1 PD-1/PD-L1 inhibitors in metastatic CRC

In the initial phase I study of MDX-1106 (anti-PD-1 antibody), irAEs were specifically concerned (Brahmer et al., 2010). In this study, 14 metastatic CRC patients were well tolerated to the maximum planned dose of 10 mg/kg. Among the 14 patients, no grade≥3 irAEs occurred. However, gastrointestinal toxicities attributed to MDX-1106 were observed. Out of 39 patients including CRC, one experienced grade 3 ascites, and one experienced grade 3 colitis. Two other patients experienced grade 2 stomatitis. None of the patients received treatment for these gastrointestinal toxicities (Brahmer et al., 2010).

The KEYNOTE-016 study reported in 2015, in which 41 patients with metastatic colorectal cancer were given treatment with pembrolizumab 10 mg/kg every 14 days, showed 40 cases (98%) of adverse events and 17 cases (41%) of grade III or higher. Special adverse reactions included thyroiditis or hypothyroidism (10%), asymptomatic pancreatitis (15%), diarrhea (24%), intestinal obstruction (7%), and upper respiratory tract infection (7%) (Le et al., 2015).

In a cohort of 20 PD-L1 positive advanced CRC patients, the irAEs of pembrolizumab treatment were systematically analyzed (O'Neil et al., 2017). The most important category of irAEs is pneumonitis including interstitial lung disease and acute interstitial pneumonitis. Pembrolizumab treatment was suggested to be held if any pneumonitis events reached grade 2 and pembrolizumab treatment was permanently discontinued if any pneumonitis events were above grade 3 (O'Neil et al., 2017). While a similar course of action was applied to hepatitis (O'Neil

TABLE 1 Summary of PD-1/PD-L1 inhibitors in clinical studies in colorectal cancer patients. mCRC, Metastatic colorectal cancer; LARC, locally advanced rectal cancer

Author	Year	Trial population	Drug	N	AE Grade1-2	AE Grade3-4
Le et al. (2015)	2015	mCRC	Pembrolizumab	41	23%	17%
Overman et al. (2017)	2017	mCRC	Nivolumab	74	49%	20%
André et al. (2020)	2020	mCRC	Pembrolizumab	149	41%	56%
Stein et al. (2021)	2021	mCRC	Avelumab	43	NA	NA
Haag et al. (2022)	2022	mCRC	Pembrolizumab	20	NA	5%
Bando et al. (2022)	2021	mCRC	Nivolumab	38	NA	NA
Brahmer et al. (2010)	2010	mCRC	MDX-1106	14	36%	0
O'Neil et al. (2017)	2017	mCRC	Pembrolizumab	23	35%	4%
Ott et al. (2017)	2017	mCRC	Pembrolizumab	24	64%	16%
Morris et al. (2017)	2017	mCRC	Nivolumab	37	NA	14%
Shamseddine et al. (2020)	2020	LARC	Avelumab	13	NA	23%
Lin et al. (2021)	2021	LARC	Camrelizumab	27	97%	27%
Hu et al. (2022)	2022	LARC	Toripalimab	34	67%	9%
Wang et al. (2022a)	2022	LARC	Toripalimab	130	NA	NA
Cercek et al. (2022)	2022	LARC	Dostarlimab	12	75%	0

et al., 2017). When grade 3 colitis, rash, uveitis, iritis, endocrine AEs, thyroid disorders, neurological AEs, or hematological AEs occurred, pembrolizumab treatment was held (O'Neil et al., 2017). Among the 23 advanced colorectal carcinoma patients treated with pembrolizumab, one patient experienced grade 4 increased blood bilirubin and pembrolizumab was discontinued as suggested (O'Neil et al., 2017).

In an open-label, multicenter, phase 2 study of Nivolumab in microsatellite instability-high/mismatch repair-deficient (MSI-H/dMMR) colorectal cancer patients (CheckMate 142), 98.6% of patients were reported with all-cause AEs. Grade 3 or 4 AEs were reported in 20.3% of patients and five (6.8%) patients discontinued treatment due to AEs. Of note, one patient who received a steroid taper for grade 3 colitis still died 10 days after their last dose (Overman et al., 2017). In the 4-year follow-up from CheckMate 142, Grade 3 or 4 AEs were reported to increase from 20.3% to 32% and AEs that lead to discontinuation increased from 6.8% to 13% (Overman et al., 2017). 5 patients discontinued treatment due to drug-related adverse events, including ALT elevation, colitis, duodenal ulcer, acute kidney injury, and stomatitis (n = 1 each) (Overman et al., 2017).

In the KEYNOTE-177 study, 153 MSI-H CRC patients in the trial group were given 200 mg of pembrolizumab every 3 weeks, and the study reported adverse reactions in 149 (97%) patients in the trial group. Common adverse reactions included diarrhea, fatigue, nausea, loss of appetite, and alopecia in 22% of grade 3 and higher adverse reactions, and immune-related adverse reactions included hypothyroidism, colitis, hyperthyroidism, pneumonia, and adrenal insufficiency in 9% of grade 3 and higher immune-related adverse reactions (André et al., 2020).

In the PICCASSO study, 20 patients with refractory colorectal cancer were treated with pembrolizumab and maraviroc (8 cycles) followed by pembrolizumab monotherapy. The study results reported that the most common adverse reactions during treatment in 20 patients were fatigue (30%), rash and pruritus (15%), and elevated AST (10%). Only one patient had a grade 3 adverse reaction, manifesting as hyperglycemia; one other patient had hypothyroidism and one patient had keratitis (Haag et al., 2022).

In a trial of pembrolizumab for a patient with recurrent carcinoma of the anal canal, four out of 24 patients developed grade 3 adverse events and continued therapy after symptomatic treatment (Ott et al., 2017). In a clinical trial of Nivolumab for a patient with metastatic anal cancer, five out of 37 patients experienced grade 3 adverse events. One patient developed grade 2 pneumonitis and subsequently received steroid therapy and a temporary treatment break while another patient received a short course of corticosteroids for the treatment of nivolumab-related autoimmune hypothyroidism (Morris et al., 2017).

2.2 PD-1/PD-L1 inhibitors in locally advanced rectal cancer

Neoadjuvant therapy for CRC is mainly aimed at locally advanced rectal cancer and some resectable metastatic CRC. Traditional neoadjuvant therapies include chemotherapy, radiotherapy, targeted therapy, and combination therapy. At present, neoadjuvant therapy for CRC is mainly radiotherapy, combined with chemotherapy drugs, and the addition of PD-1 to neoadjuvant therapy for cancer is a new attempt. In a prospective single-arm multicenter phase II trial by Shamseddine's team, mFOLFOX6 plus avelumab (10 mg/kg) was given every 2 weeks for a further 6 cycles to 13 patients with progressive colorectal cancer

who had undergone 5 cycles of total 25 Gy radiotherapy included in the study. A total of 27 adverse reactions were recorded in 13 patients, with the most common adverse reactions being diarrhea and fatigue (36%). Three grade 3 adverse events, one small bowel obstruction, one *Salmonella* colitis, and one acute kidney injury (Shamseddine et al., 2020).

In a prospective, single-arm phase II trial by Lin's team in 2021, 30 patients with locally progressive rectal adenocarcinoma were given a 5 × 5 Gy dose of radiotherapy and two 21-day treatments of CAPOX in combination with camrelizumab1 week after the start of radiotherapy, followed by radical surgery. The study results reported that the most common treatment-related adverse reactions were leukopenia (80%), and reactive cutaneous capillary endothelial hyperplasia (73%). Immune-related adverse reactions were all grade 1–2, the most common being reactive cutaneous capillary endothelial hyperplasia in 22 of 27 patients (81%); hypothyroidism was seen in two other patients (Lin et al., 2021).

In a single-center phase II study conducted in China, the participants received Toripalimab 3 mg/kg intravenously on day 1, with or without celecoxib 200 mg orally twice daily from day 1–14 of each 14-day cycle, for six cycles before surgical resection. 26 (76%) of 34 patients had at least one treatment-related adverse event during the study. The most common grade 1–2 treatment-related adverse events were hyperthyroidism (18%), fatigue (12%), increase in aspartate aminotransferase levels (12%), abdominal pain (12%), and pruritus (2%) in the combination group; and fatigue (24%), pruritus (18%), nausea (18%), and rash (18%) in the Toripalimab monotherapy group (Hu et al., 2022).

TORCH is a randomized, prospective, multicentre, double-arm, phase II trial of short-course radiotherapy (SCRT) combined with chemotherapy and immunotherapy in LARC. The consolidation arm will receive SCRT, followed by 6 cycles of capecitabine plus oxaliplatin (CAPOX) and Toripalimab. The induction arm will receive 2 cycles of CAPOX and Toripalimab, then receive SCRT, followed by 4 cycles of CAPOX and Toripalimab. Among 130 patients, the grade 3–4 immune-related toxicities were 7.7% (Wang et al., 2022a).

In a phase II study with published results in 2022, a total of 12 patients have completed treatment with dostarlimab and have undergone at least 6 months of follow-up. Adverse events of any grade occurred in 12 of the 16 patients (75%; 95% CI, 48–92). No adverse events of grade 3 or higher were reported. The most common adverse events of grade 1 or 2 included rash or dermatitis (in 31% of the patients), pruritus (in 25%), fatigue (in 25%), and nausea (in 19%). Thyroid-function abnormalities occurred in 1 patient (6%) (Cercek et al., 2022).

2.3 Management of irAEs in CRC patients

Due to the broad range of irAEs in CRC patients treated with ICI, the management of irAEs is drawing increasing attention (Darnell et al., 2020). Immune-related toxicities vary in onset, severity, and potential biology, and they may affect a wide range of organs, thus requiring specialized management approaches (Brahmer et al., 2018). Among the various irAEs, skin toxicity such as rash, pruritus and vitiligo are generally the most common and earliest to occur. Although most dermal toxicities are transient, their higher incidence is associated with patient quality of life. Gastrointestinal toxicity is also one of the most common complications. The most common clinical manifestations of immune-associated gastrointestinal toxicity range from very frequent and/or loose stools to symptoms of colitis (e.g., stool mucus, abdominal pain, fever, rectal bleeding). Compared to the first two symptoms, immunotherapy-associated pneumonia is a less frequent but potentially serious toxic adverse reaction. Moreover, immune-related endocrine adverse events occasionally occur, usually in the form of symptoms or abnormal laboratory parameters. In addition, there are some diseases with lower morbidity, including cardiovascular system, neurological system, renal system, etc. They can occur at any time during a patient's treatment, most commonly during the first 3 months of therapy. Management of irAEs is primarily focused on glucocorticoid therapy. Most symptomatic irAEs (except for endocrine disease) are treated well with several weeks of glucocorticoid therapy. In addition, although most irAEs regress, some become chronic and may require lifelong treatment such as hormone supplementation or immunosuppression (Conroy and Naidoo, 2022).

There are few relevant clinical studies, and the methods of treatment and management are mainly proposed and summarized by experienced specialists. The need for clinical management is primarily determined by the severity of the organs and irAEs involved, and management includes discontinuation of ICI therapy and initiation of topical, oral, or parenteral steroids. Steroid-related medications are currently commonly used for treatment, but the jury is still out on the optimal initial steroid dose and duration of steroid therapy, with the expectation that more prospective evidence will support this in the future. In addition, there are some expert recommendations for relatively severe irAEs, and perhaps with the use of immunosuppressive drugs.

According to the 2022 updated ESMO guidelines, irAEs management generally consists of four sequential steps: i) diagnosis and grading of irAEs, ii) ruling out differential diagnoses and pre-immunosuppression work-up, iii) selecting the appropriate immunosuppression strategy for grade 2 events and iv) active evaluation at 72 h to adapt treatment (Haanen et al., 2022). The recommendations in the guide mainly include IR-skin toxicity, IR-endocrinopathies, IR-hepatotoxicity, IR-cholangitis, IR-pancreatic toxicity, IR-gastrointestinal toxicity, IR-pulmonary toxicities, IR-rheumatological toxicity, IR-neurological toxicity, IR-cardiovascular toxicities, IR-renal toxicity, IR-major hematological toxicity and IR-ocular toxicity (Haanen et al., 2022).

Apart from the common ICI-induced irAEs, some rare, but severe and fatal, irAEs were observed in CRC patients treated with ICI. Here, we summarized rare irAEs according to clinical management in CRC patients treated with ICI. Severe necrotizing myositis was observed in CRC patients treated with nivolumab plus ipilimumab combination therapy. After discontinuation of ICI treatment, intravenous methylprednisolone combined with intravenous immunoglobulins was provided and most of the symptoms were resolved (Tauber et al., 2019). Nivolumab plus regorafenib treatment in a CRC patient resulted in immune-related keratitis (Su et al., 2022). Glucocorticoids and autologous serum were used as a diagnostic treatment and the patient recovered from irAEs after one-month treatment (Su et al., 2022). A patient with metastatic adenocarcinoma of the colon receiving atezolizumab developed acute macular neuro retinopathy, the symptom resolved after 5 weeks of oral steroids but atezolizumab treatment was discontinued and the patient died 5 months after the onset of visual symptoms (Emens et al., 2019).

Atezolizumab plus cobimetinib treatment resulted in a high incidence of treatment discontinuation for CRC patients than atezolizumab monotherapy (21% V.S. 4%) in colorectal cancer patients due to irAEs (Eng et al., 2019). Recently, a CRC patient who received tislelizumab experienced a cooccurrence of severe myasthenia gravis, myocarditis, and rhabdomyolysis (Wang et al., 2022b). Methylprednisolone and intravenous immunoglobulin therapy were applied and the patient responded well (Wang et al., 2022b).

Endocrine irAEs did not require corticosteroid therapy according to the guidelines (Panhaleux et al., 2022). However, hormone therapy facilities the recovery of endocrine disorders developed in CRC patients during ICI treatment. It has been reported that pembrolizumab caused adrenocorticotropic hormone deficiency in a cecal mucinous cancer patient and cortisol treatment was promptly effective (Bekki et al., 2020). Primary adrenal insufficiency was observed in a patient treated with nivolumab and hydrocortisone effectively corrected the hyponatremia (Deligiorgi and Trafalis, 2020). Diabetes mellitus was observed in a CRC patient treated with pembrolizumab and insulin therapy and management of electrolytes were provided (Kichloo et al., 2020). Ipilimumab and nivolumab treatment caused anterior hypophysitis in a CRC patient and stress dose IV hydrocortisone levothyroxine attenuated the symptoms. The patient was rechallenged with nivolumab monotherapy and remains asymptomatic (Jing et al., 2020).

3 Prediction of irAEs in CRC patients treated with ICI

The common adverse reactions of the antibody class of PD-1 and PD-L1 drugs currently in common use can be manifested in the skin, endocrine, gastrointestinal, and cardiac organs. Generally speaking, adverse reactions usually appear 2–3 months after drug administration, and the first manifestation is mostly seen in the skin. In summary, some common immunotherapy-related adverse reactions include fatigue, rash, colitis, hyper/hypothyroidism, anemia, decreased neutrophils, and elevated amylase. Some specific complications of immunotherapy are also of concern, including neurological, allergic, pneumonia, renal, and ocular adverse reactions, which can have very serious effects when they happen. Hence, the prediction of irAEs as well as patient monitoring would provide favorable results for patients who experienced irAEs and needed a rechallenge. Current guidelines on adverse reactions to immunotherapy focus on the identification of adverse reactions and corresponding treatment regimens, and it would certainly be more beneficial for patients to be able to predict this outcome in advance. According to the existing research, there are two main types of prediction methods, multi-omics analysis, and serological biomarkers, respectively.

3.1 Multi-omics analysis

The initial analysis of predicative biomarkers for irAEs in CRC patients is a multi-omics prediction method that analyzed mRNA, miRNA, lncRNA, and protein expression and non-silent gene mutations across 26 cancer types including rectum adenocarcinoma and colon adenocarcinoma (Jing et al., 2020). Researchers sought to identify additional predictive factors for irAEs by conducting a comprehensive screening across mRNA, miRNA, lncRNA and protein expression, and non-silent gene mutations across 26 cancer types. The results show that the lymphocyte cytosolic protein 1 (LCP1), which is involved in T-cell activation, achieved the highest correlation coefficient (Rs = 0.82, FDR = 6.69×10^{-3}, Figure 1). In the study, the authors finally came up with a bivariate regression model of LCP1 and ADPGK expression in tumor tissues that can accurately predict irAEs. This was followed by a retrospective study of cancer patients receiving anti-PD-1/PD L1 therapy at Beijing Shijitan Hospital, which culminated in a preliminary validation of the model's accuracy in the real world (Jing et al., 2020).

A pan-cancer transcriptomic analysis showed that expression levels of splicing factors were predictive of irAEs risk (He et al., 2021). The researchers detected and characterized the relationship between the expression of splicing isoforms and irAE ROR using pancancer data. The top ten irAE ROR significantly correlated splicing isoforms were utilized for building the irAE ROR predictions. Combinations between any two or three of these predictors were then evaluated by Spearman correlation and goodness of fit using the log-likelihood ratio test. Notably, the combination of CDC42EP3-206 and TMEM138-211 with most of the other predictors achieved better predictive performance (Figure 2) (He et al., 2021).

In addition, another study used a similar approach in another comprehensive analysis of cellular and molecular factors in 9,104 patients with 21 types of cancer. Researchers identified 11 new predictors of irAEs by screening global multi-omics data. Among them, IRF4 showed the highest correlation and the best predictive performance of the IRF4-TCL1A-SHC-pY317 trivariate model (Zhang et al., 2022). The genome-wide association study was also utilized to identify single nucleotide polymorphisms that are associated with the risk of irAEs (Udagawa et al., 2022).

Recently, a genome-wide association study of 1,751 patients on ICI across 12 cancer types was performed and rs16906115 near IL7 was found and replicated in three independent studies (Groha et al., 2022). Mechanically, the authors showed that patients carrying the IL7 germline variant exhibited significantly increased lymphocyte stability after ICI initiation, which was itself predictive of downstream irAEs (Groha et al., 2022).

3.2 Serological biomarkers

Serological biomarkers have long been explored to predict the incidence of irAEs due to their cheap and easy availability compared to expensive histological tests. Adam et al. found that absolute lymphocyte count was correlated with the risk of irAEs in colon cancer patients treated with nivolumab or pembrolizumab (Diehl et al., 2017). Their data suggest that patients with higher baseline lymphocyte counts have a greater risk for irAEs, whereas patients with lymphopenia at baseline and persistent lymphopenia while on therapy have a shorter time to progression on these agents (Diehl et al., 2017). The results of a study also demonstrate that peripheral blood inflammatory markers can serve as predictors of treatment response and prognosis in patients with advanced GC and CRC receiving anti–PD-1 therapy (Fan et al., 2021). It has been shown that the rate of irAEs is higher in CRC patients with low platelet-to-lymphocyte ratio patients (Fan et al., 2021).

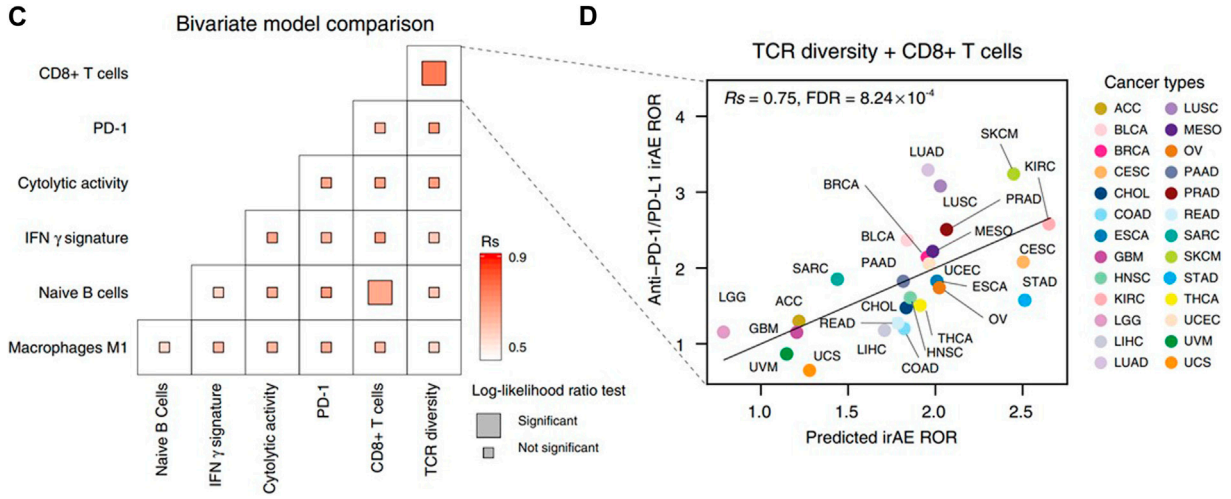

FIGURE 1
(C) Comparison of performance of bivariate models in predicting irAEs for all combinations of the top ten irAEs ROR significantly correlated genes. Spearman correlation (Rs) was calculated between the predicted and observed irAEs ROR. The shade of the square indicates the Rs, and the size indicates the significance of the log-likelihood ratio test. **(D)** Combined effect of LCP1 and ADPGK bivariate model (Spearman correlation, Rs = 0.91, FDR = 7.94 × 10^{-9}). The equation of the bivariate regression model is 0.37× LCP1 + 0.70× ADPGK−9.10. The image quoted from Nat Commun., Multi-omics prediction of immune-related adverse events during checkpoint immunotherapy, Jing Y et al., 2020 October 2; 11(1):4946 (Jing et al., 2020).

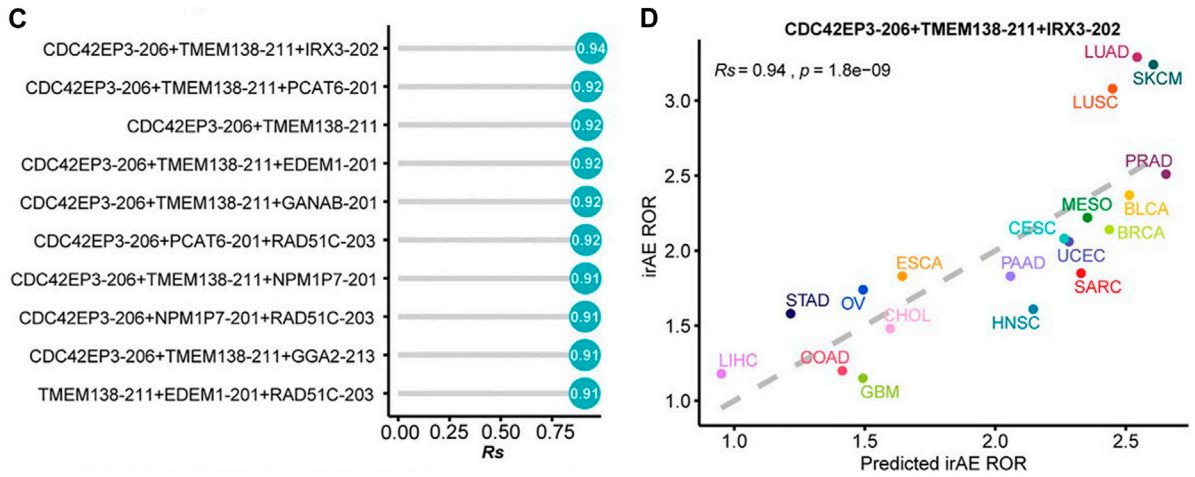

FIGURE 2
(C) Comparison of performance of bivariate and trivariate models in predicting irAEs for all combinations of the top ten irAEs ROR significantly correlated splicing isoforms. Rs was calculated between predicted and observed irAEs ROR. **(D)** Combination of CDC42EP3-206, TMEM138-211, and IRX3-202 to predict irAE risk. The dot color represents the cancer type. The dashed line represents the linear fit. The image quoted from Front Pharmacol., Pan-Cancer Analysis Reveals Alternative Splicing Characteristics Associated With Immune-Related Adverse Events Elicited by Checkpoint Immunotherapy, He X et al., 2021 November 24; 12:797852 (He et al., 2021).

In pan-cancer studies including colon cancer patients showed that a lower relative lymphocyte count, higher albumin level, and higher absolute eosinophil count were significantly associated with the occurrence of irAEs (Bai et al., 2021). Importantly, the study showed that a higher lactate dehydrogenase level was an independent predictor of irAEs of grade ≥3 (Bai et al., 2021). However, a larger validation cohort is desperately needed to verify the efficacy of these biomarkers in colorectal cancer.

In a gastrointestinal cancer cohort, serum CD28, IL-4, IL-15, and PD-L1 were significantly elevated in patients with grade 3–5 irAEs (Wang et al., 2022c). Interestingly, serum IL-6 was found higher in patients with thyroiditis and colitis. IL-22 and stem cell factor (SCF) levels were found higher in patients with colitis. IL-1a, IL-21, LIF, and PIGF-1 levels were significantly higher in patients with myositis and BTLA, GM-CSF, IL-4, PD-1, PD-L1, and TIM-3 levels were significantly higher in patients with rash (Figure 3) (Wang et al., 2022c). Since it is of special significance to predict organ-specific irAEs, this work provided a breakthrough point to make a personalized prediction of irAEs.

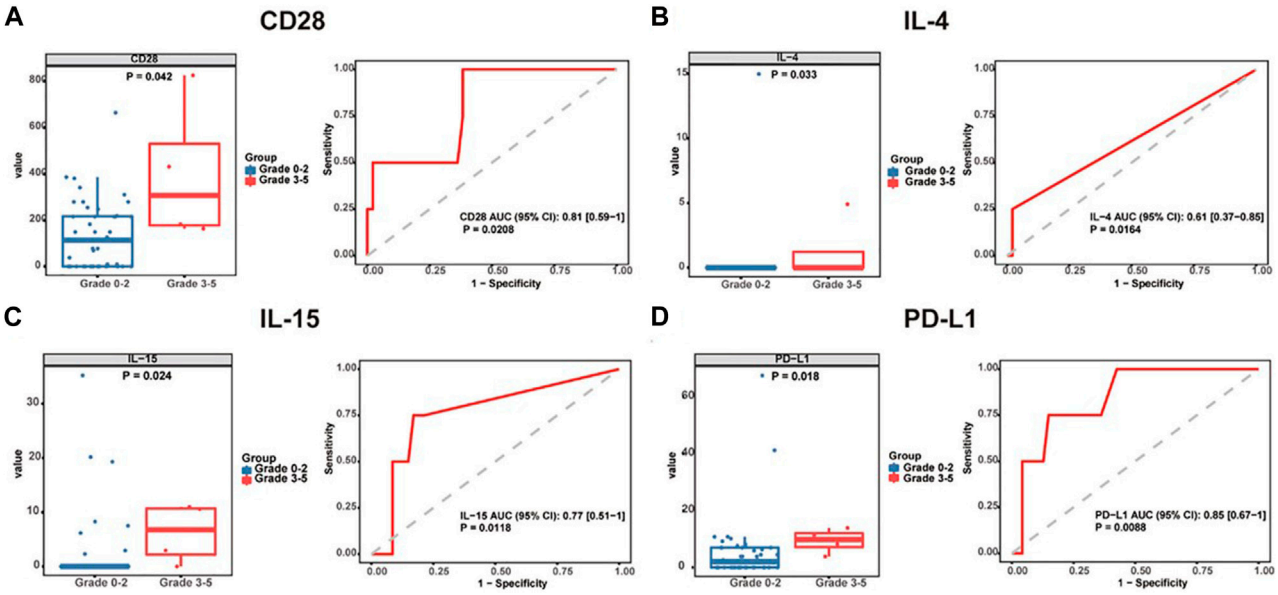

FIGURE 3
Baseline serum cytokine levels are significantly associated with irAE development and severity. Box plots (left) showing the distribution of serum cytokines **(A)** CD28, **(B)** IL-4, **(C)** IL-15, and **(D)** PD-L1 in grade 0–2 and 3-5 patients. ROC curve (right) analysis of sensitivity and specificity of serum cytokines **(A)** CD28, **(B)** IL-4, **(C)** IL-15, and **(D)** PD-L1 from baseline, to distinguish between grade 0–2 and 3-5 irAEs. The median of each group and *p*-value were calculated using the Mann-Whitney U test ($p < 0.05$). irAEs: immune-related adverse events, ROC: receiver operating characteristics. The image quoted from Front Immunol., Serological biomarkers predict immune-related adverse events and clinical benefit in patients with advanced gastrointestinal cancers, Wang Y et al., 2022 September 8; 13:987568 (Wang et al., 2022c).

There is previous evidence of a relationship between gut microbiota composition and response to treatment in patients with irAEs. The use of fecal microbiota transplantation for the treatment of colitis has also been explored and has been successfully used to treat immunotherapy-associated colitis in a series of cases17 (Wang et al., 2018). In the future, models that use gastrointestinal flora in conjunction with relevant biomarker information to predict irAEs may also be further explored.

4 Summarize

With the widespread use of PD-1 and PD-L1 drugs in various oncology areas, there is a growing body of data on the safety and efficacy studies of these drugs. Immune-related adverse events (irAEs) during anti-PD-1 or PD-L1 antibody therapy are caused by disturbances in immune activation and immune homeostasis, can affect any organ system, and in some cases can be fatal. Pneumonia is the most common fatal irAEs, with a mortality rate of 10% and accounting for 35% of anti-PD-1/PD-L1 treatment-related deaths. Myocarditis is the most fatal irAEs, with a 50% mortality rate. Therefore, predictive biomarkers of irAEs are needed to determine the benefit-risk ratio for patients receiving anti-PD-1/PD-L1 therapy. Several relevant basic studies have been performed to investigate potential predictors of irAEs risk in patients receiving anti-PD-1/PD-L1 therapy in 26 tumor types by integrating real-world pharmacovigilance and molecular-omics data. It may provide the oncology field with a way to identify potential biomarkers of irAEs in cancer immunotherapy. In the future, we look forward to more large-scale clinical data to validate the utility of these methods in the field of colorectal cancer so that we can intervene early in high-risk groups for targeted surveillance and timely individualized and balanced treatment.

Author contributions

LS contributed to the study conception and design, and critical revision of the manuscript for important intellectual content. CM and XZ contributed to the data acquisition. JZ and ZZ contributed important guidance for this study. All the authors have read and approved the final version of this 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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Potential role of gut microbes in the efficacy and toxicity of immune checkpoints inhibitors

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In recent years, Immune checkpoint inhibitors have been extensively used in the treatment of a variety of cancers. However, the response rates ranging from 13% to 69% depending on the tumor type and the emergence of immune-related adverse events have posed significant challenges for clinical treatment. As a key environmental factor, gut microbes have a variety of important physiological functions such as regulating intestinal nutrient metabolism, promoting intestinal mucosal renewal, and maintaining intestinal mucosal immune activity. A growing number of studies have revealed that gut microbes further influence the anticancer effects of tumor patients through modulation of the efficacy and toxicity of immune checkpoint inhibitors. Currently, faecal microbiota transplantation (FMT) have been developed relatively mature and suggested as an important regulator in order to enhance the efficacy of treatment. This review is dedicated to exploring the impact of differences in flora composition on the efficacy and toxicity of immune checkpoint inhibitors as well as to summarizing the current progress of FMT.

KEYWORDS

immune checkpoint inhibitors, gut microbes, efficacy, toxicity, faecal microbiota transplantation, immune-related adverse events

1 Introduction

The human intestine is populated by trillions of microbes (Bruneau et al., 2018; Wong and Yu, 2019), approximately 150–400 microbial species. It is typical that most of these species in the microbial community belong to the Bacteroidetes, Firmicutes, Actinobacteria, and Proteobacteria phyla (Davenport et al., 2017). As an essential part of the mammalian gut ecology, they play a key role in the maintenance of intestinal barrier homeostasis, the synthesis and metabolism of substances, and the immune surveillance of cancer (Yi et al., 2018; Peng et al., 2020), which is why gut microbes are also known as a "hidden organ" in humans. Roles of intestinal microbiota are diverse and may exchange upon completely different clinical backgrounds and host states. They can maintain the integrity of the intestinal barrier and enhance the immune response during immunotherapy. Nonetheless, they can also favor the proliferation of cancer cells, promote the growth and expansion of tumors and weaken the anti-tumor effect. Therefore, the dynamic identification of intestinal microbiota is of great importance for cancer immunotherapy (Chaput et al., 2017; Derosa et al., 2020).

Since the Food and drug administration (FDA) approval of the cytotoxic T-lymphocyte antigen-4 (CTLA-4) inhibitor ipilimumab provides effective treatment against metastatic melanoma in 2011 (Yi et al., 2018), a large number of drugs have entered into clinical trials and been in use. Compared with traditional tumor treatment methods (such as surgery, radiotherapy and chemotherapy), immune checkpoint inhibitors (ICIs) can significantly improve overall survival, reduce the rate of recurrence and delay the progression of tumors in patients with a variety of cancers (Zhang J. et al., 2022), which has brought unprecedented efficiency to advanced melanoma (Gopalakrishnan et al., 2018; Coutzac et al., 2020), renal cell carcinoma (Motzer et al., 2018; Tucker and Rini, 2020), non-small cell lung cancer (NSCLC) (He D. et al., 2021; Boesch et al., 2021) and other types of cancer. Currently, cancer immunotherapy has progressed rapidly and has become an important scientific breakthrough of cancer treatment, especially the application of ICIs like anti-programmed cell death protein 1/anti-programmed cell death 1 ligand 1 (anti-PD-1/PD-L1) and anti-CTLA-4. While early indications offer great hope for improving outcomes for cancer patients, ICIs are not without their limitations. What heads the list is that the response rates are quite low varying from 13% to 69% depending on the treatment regimen and cancer type (Topalian et al., 2012; Borghaei et al., 2015; Luke et al., 2017; Park et al., 2023), thus not all patients can benefit from the treatment. Moreover, complex and unpredictable immune-related adverse events (irAEs) may occur (Wang D. Y. et al., 2018), which refers a spectrum of unusual immunotherapy-related, potentially harmful, immunological reactions due to the generalized immune system over-reactivity and immune-mediated toxicities upon the use of the intravenous infusion of MAbs. Patients often experienced severe dermatitis, nephritis, hepatitis, arthritis, and other severe diseases (Stanley et al., 2016; Yahfoufi et al., 2023) Roughly one-third of recipients experienced these reactions during treatment and have no choice but to stop immunotherapy (Dubin et al., 2016; Anderson et al., 2019; Zhang J. et al., 2022; Thompson et al., 2022). Nowadays, mounting evidence shows that irAEs are similarly associated with the intestinal microbiota. Patients who developed ICI-related colitis have a relatively high abundance of Faecalibacterium and other Firmicutes while those without colitis have a high abundance of Bacteroidetes (Chaput et al., 2017). It may be possible to predict the risk of irAEs based on the intestinal microbiota composition.

How to modulate the microbiota to enhance the efficacy of ICIs and reduce the incidence of irAEs has become a hot topic of current research. Nowadays, flora transplantation in the form of capsules or fecal microbiota suspension is a more mature approach (Zhang J. et al., 2022), which can improve the stability of intestinal microbes and increase the abundance of intestinal flora to bring better prognosis for patients (Tan et al., 2022). Previously, the remarkable success of early trials treating *Clostrium difficile* infection by reconstitution of the gut microbiome is cause for measured but realistic hope (McKenney and Pamer, 2015; Smd et al., 2020). Subsequently, fecal microbiota transplantation was successfully promote response in a small number of ICIs refractory melanoma patients (Baruch et al., 2021). Therefore, this review aims to clarify the relationships between microorganisms and the efficacy and irAEs of ICIs. Additionally, we are dedicated to pointing out opinions on how to modulate microorganisms to enhance the quality of life for patients with advanced malignant tumors and reduce treatment side effects.

2 Gut microbiome modulates the efficacy of immunotherapy

2.1 Gut microbiome modulation of ICIs treatment efficacy in different types of solid tumors

In recent years, several studies have demonstrated that the composition of intestinal microbiome is associated with the efficacy of immunotherapy. Through quantitative metagenomics using next-generation sequencing, quantitative polymerase chain reaction or 16S ribosomal RNA sequencing, the researchers were able to analyze the composition of the intestinal microbiota as well as functions of microbiota which are beneficial to identify the responders who experienced immunotherapy. 16S ribosomal RNA sequencing has provided a more complete picture of the compositon of microbial inhabitants of the gut (Lamendella et al., 2012; Zhang H. et al., 2022), which based on the variable regions (V3-V4) (Whon et al., 2021). Nonetheless, the information on the functional relationships within microbial communities, or between the microbiota and the human host is very limited. Therefore, The more costly metagenomic next-generation sequencing could help identify bacteria on species level and obtain potential functional insight although a wealth of functions unknown (Hajjo et al., 2022; Zwezerijnen-Jiwa et al., 2023). To explore and understand microbial phylogenetic and functional compositions in human gut microbiota, nucleic acid sequencing can be offered. These approaches have enabled the characterization of the phylogenetic and functional microbial communities inhabiting the gut, which will be important for future diagnostic instruments for various diseases (Cong and Zhang, 2018). Nowadays, reports on the relationship between the gut microbiota and immune efficacy mainly focus on seven types of cancer, as shown in Table 1. Metastatic melanoma (MM) and non-small cell lung cancer (NSCLC) account for the highest proportion among them. These intricate interplays will be elaborated in detail following.

2.1.1 Bacterial markers for immunotherapy against metastatic melanoma

Several studies on patients with metastatic melanoma revealed that there was a significant difference in the diversity of intestinal microbiome between those who responded to anti-PD-1 treatment and those who did not. In metastatic melanoma, Firmicutes were found to be more frequent in responders. Additionally, the diversity of Bacteroidetes was notably higher among those who did not respond (Frankel et al., 2017; Gopalakrishnan et al., 2018a; Matson et al., 2018; Jin et al., 2019; Martin et al., 2019; Peters et al., 2019; Derosa et al., 2020; Peng et al., 2020; Song et al., 2020; Andrews et al., 2021; Mao et al., 2021; Fang et al., 2022; Xu et al., 2022). The Proteobacteria phylum was more commonly found in the intestinal flora of non-responders to metastatic melanoma. However, Matson et al. discovered an enrichment of *Klebsiella pneumoniae* (belonging to Proteobacteria phylum) in the feces of

TABLE 1 Studies about the relationship between the gut microbiome and response to immune checkpoint inhibitor

cancer site	author	year	ICI	sample type	accessment method	patients	R(n)	NR(n)
MM	Brandilyn A. Peters et al.	2019	Anti-PD1 Anti-CTLA4	fecal	16SrRNA+mNGS	27	**Firmicutes:** *Faecalibacterium* **Bacteroidetes:** *Parabacteroides*	**Proteobacteria:** *Bilophila* **Bacteroidetes:** *Bacteroides ovatus* **Firmicutes:** *Blautia producta, Ruminococcus gnavus*
MM	Matson et al.	2018	Anti-PD1 Ipilimumab	fecal	16SrRNA+mNGS	42	**Firmicutes:** *Enterococcus faecium, Veillonella parvula, Lactobacillus* **Actinobacteria:** *Collinsella aerofaciens, Bifidobacterium adolescentis, Bifidobacterium longum* **Proteobacteria:** *Klebsiella pneumoniae* **Bacteroidetes:** *Parabacteroides merdae*	**Firmicutes:** *Ruminococcus obeum, Roseburia intestinalis*
MM	N. Chaput et al.	2017	Ipilimumab	fecal	16SrRNA	26	**Firmicutes:** *Ruminococcus, Lachnospiraceae, Faecalibacterium*	**Bacteroidetes:** *Bacteroides*
MM	Miles Andrews et al.	2021	ipilimumab either nivolumab or pembrolizumab	fecal	16SrRNA+mNGS	77	**Bacteroidetes:** *Bacteroides stercoris, Parabacteroides distasonis* **Firmicutes:** *Fournierella massiliensis*	**Proteobacteria:** *Klebsiella aerogenes* **Firmicutes:** *Lactobacillus rogosae*
MM	Frankel et al.	2017	Ipilimumab nivolumab Pembrolizumab	fecal	mNGS	39	**Firmicutes:** *Streptococcus parasanguinis, Dorea formicigenerans* **Bacteroidetes:** *Bacteroides caccae*	**Firmicutes**: *Faecalibacterium prausnitzii, Holdemania filiformis* **Bacteroidetes:** *Bacteroides thetaiotaomicron*
MM	Rebecca C. Simpson et al.	2022	nivolumab ipilimumab	fecal	16SrRNA	103	**Firmicutes:** *Faecalibacterium prausnitzii, Butyricicoccus pullicaecorum* **Verrucomicrobia:** *Akkermansia muciniphilia*	**Bacteroidetes:** *Bacteroidaceae*
MM	V.Gopalakrishnan et al.	2018	PD1	fecal	16SrRNA+mNGS	112	**Firmicutes**: **16s:** *Clostridiales, Ruminococcaceae* **mNGS:***Faecalibacterium*	**Bacteroidetes: 16s:** *Bacteroidales* **mNGS**: *Bacteroides thetaiotaomicron* **Proteobacteria:***Escherichia coli* **Firmicutes:** *Anaerotruncus colihominis*
MM	Diwakar Davar et al.	2021	pembrolizumab	fecal	mNGS	15	**Firmicutes:** *Lachnospiraceae, Ruminococcaceae* **Actinobacteria:** *Bifidobacteriaceae, Coriobacteriaceae*	**Bacteroidetes**
NSCLC	Peng Song et al.	2020	Anti-PD1	fecal	mNGS	63	**Bacteroidetes:** *Parabacteroides* **Euryarchaeota:** *Methanobrevibacter*	**Firmicutes:** *Veillonella, Selenomonadales, Negativicutes*
NSCLC	Jin et al.	2019	Nivolumab	fecal	16SrRNA	25	**Bacteroidetes:** *Alistipes putredinis, Prevotella copri* **Actinobacteria:** *Bifidobacterium longum* **Firmicutes:** *Lachnobacterium, Lachnospiraceae* **Proteobacteria:***Shigella*	**Firmicutes:** *Ruminococcus* **Actinobacteria:** *Bifidobacterium longum* **Bacteroidetes:** *Prevotella copri*
NSCLC	Yueping Jin et al.	2019	nivolumab	fecal	16SrRNA	77	**Bacteroidetes:** *Alistipes putredinis, Prevotella copri* **Actinobacteria:** *Bifidobacterium longum*	**Firmicutes:** *Ruminococcus*
NSCLC	Chao Fang et al.	2022	nivolumab camrelizumabpembrolizumab	fecal	mNGS	85	**Bacteroidetes:** *Bacteroidesmassiliensis, prevotellaceae, Alistipes obesi*	**Firmicutes:** *Enterocloster* **Bacteroidetes:** *Bacteroides fragilis*

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TABLE 1 (*Continued*) Studies about the relationship between the gut microbiome and response to immune checkpoint inhibitor

cancer site	author	year	ICI	sample type	accessment method	patients	R(n)	NR(n)
NSCLC	Taiki Hakozaki et al.	2021	nivolumab, pembrolizumab, or atezolizumab	fecal	16SrRNA	70	**Firmicutes:** *Ruminococcaceae UCG 13, Agathobacter,Lachnospiraceae UCG001*	NA
NSCLC	Rachel C. Newsome et al.	2022	Anti-PD1 Anti-CTLA4	fecal	16SrRNA	65	**Firmicutes:** *Ruminococcus, Faecalibacterium* **Verrucomicrobia:** *Akkermansia*	NA
NSCLC,RCC	Routy et al.	2018	Anti-PD1	fecal	mNGS	78	**Verrucomicrobia:** *Akkermansia muciniphila* **Firmicutes:** *Ruminococcus,Eubacterium* **Bacteroidetes:** *Alistipes*	NA
Thoracic-carcinoma	Huihui Yin et al.	2021	Anti-PD1	fecal	16SrRNA	42	**Verrucomicrobia:** *Akkermansiaceae* **Firmicutes:** *Enterococcaceae, Carnobacteriaceae, Clostridiales Family XI bacterial families* **Proteobacteria:** *Enterobacteriaceae*	NA
RCC	Lisa Derosa et al.	2020	nivolumab	fecal	mNGS	58	**Bacteroidetes:** *Alistipes senegalensis, Bacteroides salyersiae* **Firmicutes:** *Clostridium ramosum* **Verrucomicrobia:** *Akkermansia muciniphila*	**Firmicutes:** *C. hathewayi, Clostridium clostridioforme*
HCC	Jinzhu Mao et al.	2021	Anti-PD1	fecal	mNGS	65	**Bacteroidetes**: *Alistipes sp Marseille-P5997* **Firmicutes:** *Ruminococcus calidus, Erysipelotichaceae bacterium-GAM147, Lachnospiraceae bacterium-GAM79*	**Firmicutes:** *Veillonellaceae*
HCC	Lili LI et al.	2020	Anti–PD-1	Buccal+fecal	16SrRNA	65	**Firmicutes:** *Clostridiales, Ruminococcaceae*	**Bacteroidetes:** *Bacteroidales*
HCC	Zheng et al.	2019	camrelizumab	fecal	mNGS	8	**Firmicutes:** *four Lactobacillus species (L. oris, L. mucosae, L.gasseri, and L. vaginalis), Streptococcus thermophilus* **Actinobacteria:** *Bifidobacterium dentium*	NA
GICA	Peng et al.	2020	Anti–PD-1 CTLA-4 blockade	fecal	16SrRNA+mNGS	74	**Verrucomicrobia:** *Akkermansia* **Bacteroidetes:** *Prevotellaceae, Prevotella/Bacteroides, Parabacteroids* **Firmicutes:** *Lachnoclostridium, Lachnospiraceae, Ruminococcaceae, Flavonifractor(Eubacterium),Dialister*	**Bacteroidetes:** *Bacteroides, Parabacteroides* **Firmicutes:** *Coprococcus, Subdoligranulum*
ESCC	Liwei Xu et al.	2022	camrelizumab	fecal	16SrRNA	46	**Bacteroidetes:** *Barnesiellaceae, Odoribacteraceae, Butyricimonas, Prevotella, Barnesiella, Odoribacter* **Synergistetes:** *Dethiosulfovibrionaceae, Pyramidobacter genus*	**Proteobacteria:** *Aeromonadales, Pseudomonadales, Moraxellaceae, Rhodocyclales, Rhodocyclaceae, Acinetobacter* **Fimicutes:** *Dialister* **Deinococcus-Thermus:** *Deinococci*
Pan-carcinoma	Zhaozhen Wu et al.	2022	Anti-PD1	fecal	mNGS	27	**Bacteroidetes:** *Parabacteroides* **Firmicutes:** *Clostridia bacterium UC5.1_2F7* **Actinobacteria:** *Bifidobacterium dentium*	**Bacteroidetes:** *Bacteroides dorei* **Actinobacteria:** *Nocardia*

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patients who responded to programmed cell death protein 1(PD1) treatment. Actinobacteria and Verrucomicrobia phylum were the only ones present in the intestinal flora of metastatic melanoma patients who responded to immunotherapy (Matson et al., 2018; Davar et al., 2021; Simpson et al., 2022), suggesting that these may be the dominant bacteria in responders. It is unclear, however, how the specific dominant phyla in metastatic melanoma may influence tumor immune effects in patients.

The appearance of paradox may be associated with microbiota-derived metabolites, such as those produced by *Clostridales* in the Fimicutes phylum and *Akkermansia muniphilla* in the Verrucomicrobia phylum (Louis et al., 2014; Morrison and Preston, 2016; Martin-Gallausiaux et al., 2021). These metabolites may enhance or diminish antitumor efficacy through immunoregulation. Favorable metabolites include short chain fatty acids, polysaccharide A, inosine, polyamines, long chain fatty acids, tryptophan derivatives and trimethylamine N-oxide. For example, Short chain fatty acids (SCFAs) are products of fiber fermentation by intestinal bacteria, which contain acetic acid, propionic acid, butyric acid, valerate and so on. SCFAs can provide energy for the colon cells and inhibit various cancer signaling pathways and inflammatory responses (Donohoe et al., 2011; Chen et al., 2019), such as the NF-κB and its downstream pathways to reduce the release of inflammatory factors (Trompette et al., 2018; He Y. et al., 2021; Zhang J. et al., 2022). Among them, Butyric acid produced by *prausnitzii* can promote the proliferation of $CD8^+$T and enhance anti-tumor immunity (Bachem et al., 2019). Mucin synthesis can be induced and intestinal mucosal integrity can be maintained on the basis of SCFAs (Guo and Li, 2019). In addition, SCFAs can stimulate DNA mismatch repair genes to increase the ability of gene expression and promote gene stability, which can also induce differentiation and apoptosis of colorectal cancer cells (Sun and Zhu, 2018). Thus, SCFAs have the potential to be used as biomarkers for the efficacy of immunotherapy (Nomura et al., 2020). Another study found Polysaccharide A (PSA), which is secreted by *Bacteroides fragilis* in the colon, can activate $CD4^+$T and promote the release of IL-10 to suppress inflammation (Wang et al., 2006; Round et al., 2011). The metabolites of *Bifidobacterium pseudobifidum* and *A. muciniphila*——inosine can bind to A2A receptors on the surface of T cells to enhance antitumor immunity and enhance the efficacy of ICIs (Mager et al., 2020). It happens that there is a similar case that Hai Wang et al. found that the trimethylamine N-oxide produced by *Clostridiales* can enhance the efficacy of immunotherapy in triple-negative breast cancer, which is proportional to $CD8^+$T cell (Wang et al., 2022). While adverse metabolites contain N-nitroso compounds, bile acids, ammonia, phenols, hydrogen sulfide, lipopolysaccharide and so on. Lipopolysaccharide is the metabolite of Gram-negative bacterial, which can promote immune escape in CRC cells through the activation of TLR4 and the induction of immunosuppressive factors (Li et al., 2014). Ammonia, phenols, and hydrogen sulfide create chronic inflammation and induce DNA damage leading to CRC development, the same as N-nitroso compounds (Ijssennagger et al., 2016; Borzì et al., 2018; Mizutani et al., 2020). Consequently, we can conclude that metabolic approach can suggest potentials in personalized management through helping prediction of efficacy process of immunotherapy.

2.1.2 Effects of the gut microbiota on non-small cell lung carcinoma

In patients with non-small cell lung carcinoma, both the Firmicutes phylum and the Bacteroidetes phylum are present in both responders and non-responders. *Bifidobacterium longum* in the Actinobacteria phylum and *A. muciniphila* in the Verrucomicrobia are beneficial bacteria that are enriched in immune responders (Routy et al., 2018a; Jin et al., 2019). *Bifidobacterium* has immunomodulatory effects and is closely related to the energy metabolism of regulatory T cells, which may improve the symptoms of colitis through the accumulation of conjugated linoleic acid (Zhou et al., 2022). *A. muciniphila* can produce inosine, induce the expression of TH1 regulatory genes in $CD4^+$ T cells (Zhang et al., 2019), and reverse PD-1 blockade by IL-12 from dendritic cells, increasing the recruitment of $CCR9^+$ $CXCR3^+$ $CD4^+$ T lymphocytes to the tumor microenvironment to kill tumor cells (Routy et al., 2018b). It has been found to be abundant in NSCLC, MM, GI tumors, and renal cell cancer responders, making it a potential microbial marker of response to immune checkpoint therapy[54]. Akkermansia muciniphila may also have epidemiological links to inflammation (Derosa et al., 2022), reduce obesity and its complications (Zhou et al., 2020), alleviate neurodegenerative diseases (Blacher et al., 2019) and inhibit premature aging (Bárcena et al., 2019).

2.1.3 Potential role of gut microbiota on other types of cancers

In patients with hepatocellular carcinoma and renal cell carcinoma, the Firmicutes phylum was more abundant in the fecal flora of patients who responsed to immunotherapy, while the Bacteroidetes phylum was relatively abundant in the fecal flora of those who did not respond (Routy et al., 2018a; Derosa et al., 2020; Li and Ye, 2020; Mao et al., 2021). Additionally, *bifidobacteria* was only found in the feces of patients with hepatocellular carcinoma patients who responded (Zheng et al., 2019), and *A. muciniphila* was only found in the feces of renal cell carcinoma patients (Routy et al., 2018a; Derosa et al., 2020), These findings suggest that the Firmicutes, Actinobacteria, and Verrucomicrobia phyla may be indicator markers for both cancers, providing valuable insight into the efficacy and prognosis of immunotherapy. Unfortunately, the gut microbiota is dynamic and evolves with the pathology. Confounding environmental factors may influence the composition of it, such as diet, medication, smoking and other lifestyle factors (Huxley et al., 2009; Conlon and Bird, 2015). So we shall make the best of our ability to control these factors including patient demographics (sex, age, race, comorbidities) (Gong et al., 2019). Besides, the same bacteria in distinct communities can have different functions in the interation with the host, which may predict contradictory prognosis. Hence, large cohorts, and clinical trials should be performed to assess the impact of gut microbiota on the effectiveness of ICIs (Rezasoltani et al., 2021; Roviello et al., 2022).

Similarly, there is a lack of literature on the relationship between immunotherapy efficacy and intestinal flora in gastrointestinal tract tumors. Peking University Cancer Hospital studied the changes in the flora of 74 GI tract tumor patients before and after treatment with immune checkpoint inhibitors and found that the composition of the patients' body flora and gut microbial metabolites affect the

patients' response to programmed cell death protein 1/programmed cell death 1 ligand 1(PD1/PDL1) antibodies. Specific response groups exhibited high abundance of Prevotella, Ruminococcaceae and Lachnospiraceae, all of which belong to the Firmicutes phylum. Additionally, *Eubacterium, Lactobacillus and Streptococcus* in different GI tumor types were positively correlated with the therapeutic response to PD1/PDL1 inhibitors. Furthermore, *Blue-green algae,* Lachnospiraceae*, Ruminococcus and Microbacterium* were all enriched in patients benefiting from colorectal cancer immunotherapy. This study highlights that gut microbes can predict response efficacy and can serve as potential biomarkers of response to immune checkpoint inhibitors. Liwei Xu et al. found a special phylum—Synergistetes, which were abundant in clinical responders of esophageal squamous cell carcinoma. Synergistetes is a rare class of anaerobic bacteria (McCracken and Nathalia Garcia, 2021) and have frequently been reported in the human oral cavity at sites of dental disease, especially periodontitis. Although Synergistetes are pathogenic, they favored the efficacy of immunotherapy in patients, thus more clinical studies and trials are needed to verify this. Moreover, Emerging evidence points that the alpha diversity is not necessarily a positive correlation with the immunotherapeutic efficacy. Huihui Yin et al. discovered that patients with a higher commensal bacterial abundance had a prolonged progression-free survival (PFS) (Yin et al., 2021). While another study did not observe statistically significant differences in bacterial taxa relative abundance between responders and non-responders. The interpretability of findings may originate from the variation of each study design and the data analyses (Peng et al., 2020). The Akkermansiaceae, Enterococcaceae, Carnobacteriaceae, and Clostridiales Family XI were all over-represented at diagnosis in patients with longer PFS (Yin et al., 2021). These studies highlight that gut microbes can predict response efficacy and can serve as potential biomarkers of response to immune checkpoint inhibitors. Therefore, the composition of intestinal microbiome plays a key role in cancer immunotherapy.

2.2 Intricate interplay between the gut microbiota and differential immunotherapeutic efficacy

Based on our statistical study, we found that Firmicutes were present in the fecal flora of responders of 19 reports across 23 studies, Bacteroidetes were present in the fecal flora of responders in 14 studies, and Actinobacteria phylum was found to have significant immune efficacy. Proteobacteria phylum, however, is controversial in its contribution to immune efficacy. *Klebsiella pneumonia*, *Shigella* and Enterobacteriaceae in Proteobacteria phylum were reported to be present in the gut microbiome of patients with responders (Matson et al., 2018; Jin et al., 2019; Yin et al., 2021). However, Liwei Xu, Brandilyn A. Peters, Miles Andrews et al. all discovered that Proteobacteria phylum was widely present in the feces of non-responding patients in their studies (Gopalakrishnan et al., 2018a; Peters et al., 2019; Xu et al., 2022). Six orders of Proteobacteria were associated with non-responders, including Aeromonodales, Pseudomonadales, Moraxellales, Rhodocyclales, Desulfovibrionales, and Enterobacterales, and were associated with shorter progression-free survival and impaired antitumor immune responses mediated by limited intratumoral lymphoid and weakened antigen presentation capacity (Gopalakrishnan et al., 2018a). The exact mechanisms by which this occurs remain unclear, and more evidence is needed to explore it. In conclusion, Verrucomicrobia, Euryarchaeota, and Synergistetes were only present in patients with responders, while Deinococcus-Thermus was present in patients without responders, as detailed in Table 2. Therefore, according to the above researches, Fimicutes, Bacteroidetes, Verrucomicrobia, Euryarchaeota, and Synergistetes phylum may be the potential biomarkers for cancer immunotherapy.

2.3 Animal testing to verify the interplay between gut microbiome and host immunity

Based on the above studies, we found modulating intestinal flora can affect the efficacy of immune checkpoint inhibitors. To a certain degree, several animal studies have now demonstrated that intervention of intestinal flora can enhance the treatment of immune checkpoint inhibitors. Yoon et al. (2021) combined Bifidobacterium shortum and PD1 inhibitors in mice and found that both $CD8^+$ T cell levels and $CD8^+$/Treg ratios were elevated in mice, increasing the anti-tumor efficacy of mice (Yoon et al., 2021). Similarly, Montalban-Arques et al. used PD1 inhibitors along with a mixture of four *Clostridium* species instilled into the stomachs of mice and found that $CD8^+$ T cells were infiltrated around the tumor tissue. As a result, this combination treatment cleared almost all tumor cells (Montalban-Arques et al., 2021) and achieved a better synergistic effect. However, all of the above are animal trials and more clinical trials are needed to explore and validate.

3 Gut microbiota in immune-related toxicity

Although immunotherapy has brought a revolutionary breakthrough in cancer treatment, the use of CTLA4 and PD1 blockers can lead to an over-activation of the immune system, resulting in increased intestinal permeability and loss of intestinal barrier integrity, which can cause systemic inflammation and immune-related adverse events (irAEs). Thus, the benefits associated with ICIs come at the cost of irAEs, and the increased efficacy is usually accompanied by irAEs. Unlike typical chemotherapy-related toxicity, it can be considered of off-target effects of an over-activated immune system (F et al., 2019), immune-related adverse events often manifest as immune-associated colitis (Liu Z. et al., 2021), diarrhea (Kelly-Goss et al., 2022), rash (Dimitriou et al., 2019), arthritis (Kostine et al., 2021) and so on (Stanley et al., 2016). Higher abundance of gut microbiota has been observed in patients experiencing mild diarrhea compared to those with severe diarrhea, suggesting that enrichment of the gut microbiota is important for the prevention of irAEs.

3.1 The gut microbiome and irAE occurrence: a new adventure world

Studies on flora and immune-related adverse events focused on five solid tumors (Table 3), in detail, patients without irAEs or with irAEs

TABLE 2 Gut microbiome bacteria in responders and non-responders to immune checkpoint inhibitors, by phylum.

Responders	Phylum							
	Firmicutes	Bacteroidetes	Actinobacteria	Proteobacteria	Verrucomicrobia	Euryarchaeota	Synergistetes	Deinococcus-Thermus
Yes	*Agathobacter*, *Butyricicoccus pullicaecorum*, Carnobacteriaceae, *Clostridiales*, *Clostridiales Family XI bacterial families*, *Clostridia bacterium UC5.1_2F7*, *Clostridium ramosum*, *Dialister*, *Dorea formicigenerans*, Enterococcaceae, Erysipelotichaceae *bacterium-GAM147*, *Enterococcus faecium*, *Eubacterium*, *Faecalibacterium prausnitzii*, *Fournierella massiliensis*, *Flavonifractor*, Lachnospiraceae *bacterium-GAM79*, Lachnospiraceae, *Lachnobacterium*, Lachnospiraceae *UCG001*, *Lactobacillus*, *Lachnoclostridium*, *Ruminococcus*, *Ruminococcus calidus*, Ruminococcaceae, Ruminococcaceae *UCG 13*, *Streptococcus parasanguinis*, *Streptococcus thermophilus*, *Veillonellaparvula*	*Alistipes obesi*, *Alistipes putredinis*, *Alistipes senegalensis*, *Alistipes sp Marseille-P5997*, *Bacteroides caccae*, *Bacteroides massiliensis*, *Bacteroides stercoris*, *Bacteroides salyersiae*, Barnesiellaceae, *Barnesiella*, *Butyricimonas*, Odoribacteraceae, *Parabacteroides merdae*, *Prevotella copri*, Prevotellaceae, *Parabacteroides distasonis*	*Bifidobacterium adolescentis*, *Bifidobacterium longum*, *Bifidobacterium dentium*, Bifidobacteriaceae, Coriobacteriaceae, *Collinsella aerofaciens*	*Klebsiella pneumoniae*, *Shigella*, Enterobacteriaceae	*Akkermansia muciniphila*	*Methanobrevibacter*	Dethiosulfovibrionaceae, *Pyramidobacter*	—
No	*Anaerotruncus colihominis* *Blautia producta*, *Coprococcus* *C.hathewayi*, *Clostridium clostridioforme*, *Dialister*, *Enterocloster*,	*Bacteroides*, *Bacteroidales*, *Bacteroidesthetaiotaomicron*, Bacteroidaceae, *Bacteroides ovatus*, *Bacteroides fragilis*, *Bacteroides dorei*, *Prevotella copri*, *Parabacteroides*	*Bifidobacterium longum*, *Nocardia*	*Aeromonadales*, *Acinetobacter*, *Bilophila*, *Klebsiella aerogenes*, Moraxellaceae, *Rhodocyclales*, *Pseudomonadales*, Rhodocyclaceae	—	—	—	*Deinococci*

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TABLE 2 (*Continued*) Gut microbiome bacteria in responders and non-responders to immune checkpoint inhibitors, by phylum.

	Phylum							
	Firmicutes	Bacteroidetes	Actinobacteria	Proteobacteria	Verrucomicrobia	Euryarchaeota	Synergistetes	Deinococcus-Thermus
Responders	*Faecalibacterium prausnitzii, Holdemania filiformis, Lactobacillus rogosae, Negativicutes, Ruminococcus obeum, Roseburia intestinalis, Ruminococcus gnavus, Subdoligranulum, Selenomonadales,* Veillonellaceae							

showed an abundance of 7 abundant bacteria in the phylum level (Table 4). The study found that the Firmicutes phylum was associated with a high probability of adverse events with immunotherapy, while the Bacteroidetes phylum was associated with a low probability of immune-related adverse events. Of the 9 articles studied, 6 articles found Firmicutes to be enriched in groups with immune-related adverse events, while Bacteroidetes phylum was similarly found in groups without immune-related adverse events. On the contrary, Mao et al. conducted a metagenomic analysis of stools from 65 patients with hepatocellular carcinoma with different responses and found that immune-associated colitis was largely associated with low diversity and abundance of gut microorganisms. Bacteroidetes phylum was found to cause more severe immune-related adverse events and is a potential biomarker for predicting severe diarrhea and colitis, while the high abundance and diversity of Firmicutes phylum may be a protective factor against immunotherapy-induced toxicity (Mao et al., 2021). However, the exact mechanism of this is still unknown and requires further research to be proven.

The abundance of Proteobacteria was significantly higher in the irAEs group compared to the no-irAEs group. Additionally, Actinobacteria, Verrucomicrobia, Acidobacteria and Synergistetes phylum were only present in the fecal flora of patients with immune-related adverse events. This could be used as a potential marker to differentiate between irAEs and no-irAEs, as detailed in Table 4.

3.2 Clinical evidence linking bacterial biomarkers to different types of irAEs

To identify specific microbial biomarkers that can be used to classify patients with mild irAEs or severe irAEs, we found that the abundance of Firmicutes、Bacteroidetes, Actinobacteria and Proteobacteria were similar between the two groups, However, patients with severe irAEs had a visible abundance of Acidobacteria, while those with grades 1-2 irAEs had a higher abundance of Synergistetes at the phylum level, as detailed in Table 5. Therefore, these statistics suggested that patients with severe irAEs had an intestinal microbial community significantly different from those with mild irAEs.Wenhui Liu et al. the Bryan-Curtis intragroup distance of the no irAE group was smaller than both the mild irAEs and severe irAEs groups, however, there was no significant difference in α-diversity among them (Liu W. et al., 2021). These studies indice that patients without irAEs have a distinctly different gut microbial composition from those with mild and severe irAEs. And the compositions of microbiome could be used to be clinical tools to stratify patients during the treatment with checkpoint blockade therapy into groups with high and mild risk of irAEs. It is very valuable for surgeons to weigh the potential danger and advantages of immunotherapy. Further research is needed to explore and validate whether the regulation of the gut microbiome affects a variety of immune-mediated adverse events (Mao et al., 2021).

4 Clinical application and potential challenges in modulating the gut microbiota

Faecal microbiota transplantation (FMT) is a process in which stools from healthy donors or previous stools from the same individual

TABLE 3 Studies that access the composition of the gut microbiome with irAEs or without irAEs

cancer site	author	time	method	sample type	irAEs	no irAEs
MM	Krista Dubin et al.	2016	16S rRNA	fecal	*Low Bacteroidaceae*	**Bacteroidetes:** *Bacteroidaceae, Rikenellaceae, Barnesiellaceae*
MM	chaput et al.	2017	16S rRNA	fecal	**Firmicutes:** *Lachnospiraceae, Ruminococcacea* **Bacteroidetes:***Bacteroidaceae*	**Bacteroidetes:** *Prevotellaceae Bacteroidaceae, Porphyromonadaceae* **Firmicutes:** *Ruminococcaceae*
UCC	Daniel Y. Wang et al.	2018	16S rRNA	fecal	**Proteobacteria:** *Escherichia* **Firmicutes:** *Clostridia*	NA
HCC	Jinzhu Mao et al.	2021	16S rRNA	fecal	**Bacteroidetes**	**Firmicutes**
MM	Miles Andrews et al.	2021	16S rRNA+mNGS	fecal	**Bacteroidetes:** *Bacteroides intestinalis* **Firmicutes:** *Intestinibacter bartlettii*	**Firmicutes:** *Dorea formicigenerans*
NSCLC	Taiki Hakozaki et al.	2021	16S rRNA	fecal	3-4: **Firmicutes:** *Agathobacter*1-2: **Verrucomicrobia**: *Akkermensia* **Firmicutes:** *Lactobacillaceae* **Proteobacteria:** *Raoultella*	**Firmicutes:** *Lactobacillaceae* **Proteobacteria:** *Raoultella*
Pan-carcinoma	Wenhui Liu et al.	2021	16S rRNA	fecal	3-4:**Bacteroidetes**:*Spirosomaceae* **Firmicutes:** *Thermoanaerobacteracea*,*Streptococcus* **Proteobacteria:** *Anaplasmataceae, Vibrionales, Stenotrophomonas* 1-2: **Firmicutes:** *Faecalibacterium, unidentified_ Lachnospiraceae* **Actinobacteria:** *Nocardiaceae* **Proteobacteria:** *Pseudomonadaceae*	**Bacteroidetes**:*Balneolales* **Proteobacteria:** *Pseudomonadales*
MM	Rebecca C. Simpson et al.	2022	16S rRNA	fecal	**Bacteroidetes:** *Bacteroidaceae*	**Firmicutes:** *Faecalibacterium prausnitzii, Oscillospira, Ruminococcus bromii, Lachnospiraceae*
ESCC	Liwei Xu et al.	2022	16S rRNA	fecal	≥3:**Firmicutes:** *Succiniclasticum, Staphylococcus* **Actinobacteria:** *Nakamurella, Actinosynnemataceae, Lentzea, Pseudonocardia* **Proteobacteria:** *Rhizobium, Chelativorans, Phyllobacteriaceae, Pelagibacteraceae, Coxiellaceae Acidicapsa, Plesiomonas* **Acidobacteria:** *Granulicella, Acidobacteriaceae, bacterium Ellin6075* **Bacteroidetes:** *Aquirestis, Flavisolibacter, Dysgonomonas* 1-2: **Firmicutes:** *Phascolarctobacterium, Anaerotruncus* **Bacteroidetes:** *Odoribacteraceae,Odoribacter, Butyricimonas* **Synergistetes:** *Synergistia, Synergistales, Synergistes* **Proteobacteria:** *Deltaproteobacteria*	NA

MM: metastatic melanoma; UCC: urothelial cell carcinoma; HCC: hepatocellular carcinoma; NSCLC: non-small cell lung carcinoma; ESCC: esophageal squamous cell carcinoma; NA: not assessed; 16S rRNA:16S ribosomal RNA sequencing; mNGS:metagenomic next generation sequencing

are transplanted into the gastrointestinal tract of recipients to balance or restore gut microbial composition (Tan et al., 2022).

4.1 FMT to boost the clinical efficacy of immunotherapy and mitigate immune-related adverse events

In recent years, the emergence of resistance to immunotherapy and the occurrence of immune-related adverse events have posed great challenges for clinical immunotherapy. Several studies suggest that modulating intestinal microbiome can enhance immunotherapy response and reduce the occurrence of complications. Fecal microbiome transplantation is a relatively mature method to regulate microbiome and restore the richness of the recipient's intestinal microbiome. Nowadays, there are three forms of faecal microbiota transplantation, including transfusion, oral administration or injection based on the capsules or manufactured bacterial fluids in order to reshape the vivo intestinal microecology, as shown in the Figure 1. Diwakar Davar et al. found that in FMT transplant-responding advanced melanoma patients circulating IL-8 downregulates. IL-8 is an immunosuppressive cytokine secreted by intratumoral and circulating myeloid cells, which correlates with poor prognosis with anti-PD1 use (Sanmamed et al., 2017; Schalper et al., 2020; Davar et al., 2021). Additionally, IL-8 was negatively correlated with increased levels of the beneficial bacteria *Faecalibacterium prausnitzii* and *A. muciniphila* in responders. Thus, FMT may adjust intestinal microecology and optimize immunotherapy, which can enhance the quality of life for patients with advanced malignant tumors and prolong their survival. Similarly, in an experiment with mice, mice that transplanted fecal microbiome from patients who had responded to anti-PD1 treatment were more active to immunotherapy and had a higher density of $CD8^+$T cells after receiving treatment while those receiving stool from non-responsive patients developed resistance

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TABLE 4 Gut microbiome bacteria in patients with irAEs or without irAEs, by phylum.

irae	Phylum						
	Firmicutes	Bacteroidetes	Proteobacteria	Actinobacteria	Verrucomicrobia	Acidobacteria	Synergistetes
Yes	*Anaerotruncus, Agathobacter, Clostridia, Faecalibacterium, Intestinibacter bartlettii*, Lachnospiraceae, Lactobacillaceae, Ruminococcaceae, *Streptococcus, Succiniclasticum, Staphylococcus, Thermoanaerobacteracea, Phascolarctobacterium*	*Aquirestis*, Bacteroidaceae, *Bacteroides, Butyricimonas intestinalis, Dysgonomonas, Flavisolibacter*, Odoribacteraceae, *Odoribacter*, Spirosomaceae	Anaplasmataceae *Acidicapsa, Chelativorans*, Coxiellaceae *Plesiomonas, Deltaproteobacteri, Escherichia*, Pseudomonadaceae, Phyllobacteriaceae, Pelagibacteraceae, *Raoultella, Rhizobium, Stenotrophomonas, Vibrionales*	Actinosynnemataceae, *Lentzea*, Nocardiaceae, *Nakamurella, Pseudonocardia*	*Akkermensia*	*Granulicella*, Acidobacteriaceae, *bacterium Ellin6075*	*Synergistia, Synergistales, Synergistes*
No	*Doreaformicigenerans, Faecalibacterium prausnitzii*, Lachnospiraceae, Lactobacillaceae, *Oscillospira Ruminococcus bromii*, Ruminococcaceae	Bacteroidaceae, *Balneolales* Rikenellaceae, Barnesiellaceae, Prevotellaceae Porphyromonadaceae	*Pseudomonadales, Raoultella*	—	—	—	—

to ICIs (Gopalakrishnan et al., 2018b). According to the researches, FMT can provide a new therapeutic opportunity for patients with solid tumors who are resistant or less effective in immunotherapy. Moreover, FMT can be used to alleviate the irAEs during treatment. Yinghong Wang et al. reported the first successful ICI-associated colitis treatment case treated with fecal microbiome transplantation (FMT) in the University of Texas MD Anderson Cancer Center in 2018 (Wang Y. et al., 2018). With early insights into potential mechanisms, they revealed that FMT can be used to modulate the gut microbiome and improved symptoms of refractory ICI-associated colitis rapidly and significantly. Subsequently in 2020, National Comprehensive Cancer Network (NCCN) guidelines introduced FMT as an optional treatment for colitis refractory to immunosuppressant therapy based on institutional availability and expertise (Ianiro et al., 2020). Although early insights into the treatment of refractory colitis are provided, the study cohorts are very small and there are significant limitations. Given the widespread application of ICI across different cancer types, It is anticipated that there may be increasing incidence of ICI-associated colitis and other irAEs. Therefore, it is essential to carry out more investigations to assess the effectiveness of FMT and further mechanistic insight should be provided.

4.2 Limitations and risks of FMT

Although FMT has the advantage of increasing the chance of obtaining a long-term reset of the microbiome, it is important to note that there are some limitations and risks associated with the transfer of pathogenic microorganisms. In 2019, Zachariah DeFilipp et al. found that two patients who underwent FMT developed extended-spectrum beta-lactamase (ESBL)–producing *Escherichia coli* bacteremia, and one of them died soon (DeFilipp et al., 2019). Additionally, a systematic review reported five patients who developed infections after FMT (Shogbesan et al., 2018). Furthermore, the emergence of COVID-19 in the last 3 years has posed a challenge for fecal microbiome transplantation, as the virus has been detected in the stool of some asymptomatic infected individuals in a research (Nagy et al., 2020; Thompson et al., 2020).

The ineffectiveness of FMT may be due to several factors, such as a decrease in the patient's immunity, the absence of taxa needed for therapy effectiveness in the FMT, and the disruption of the host microorganism due to graft failure (Davar et al., 2021). Therefore, it is essential to be aware of the potential risks and limitations of FMT.

4.3 Administration of FMT

The safety of FMT should be the primary consideration in clinical decision-making and more clinical studies should be carried to ensure the efficacy, particularly among immune-compromised patients. Additionally, the patient's commensal background should be considered before receiving FMT, as primary intestinal mucosal commensal bacteria may interfere with the colonization of the complementary flora (Zmora et al., 2018). Furthermore, it is necessary to control the types and content of beneficial bacteria used for FMT materials and the management of probiotics to produce standardized specimens and minimize potential contamination (Pierrard and Seront, 2019). Last but not

TABLE 5 Gut microbiome bacteria in patients with 1-2 irAEs and 3-4 irAEs, by phylum.

irae	Firmicutes	Bacteroidetes	Actinobacteria	Proteobacteria	Acidobacteria	Synergistetes
3-4	*Agathobacter*, *Succiniclasticum*, *Staphylococcus*, *Streptococcus*, *Thermoanaerobacteracea*	*Aquirestis*, *Dysgonomonas*, *Flavisolibacter*, Spirosomaceae	Actinosynnemataceae, *Lentzea*, *Nakamurella*, *Pseudonocardia*	*Acidicapsa*, Anaplasmataceae, *Chelativorans*, Coxiellaceae, Phyllobacteriaceae, Pelagibacteraceae, *Plesiomonas*, *Rhizobium*, *Stenotrophomonas*, *Vibrionales*	Acidobacteriaceae, *bacteriumEllin6075Granulicella*	—
1-2	*Anaerotruncus*, *Faecalibacterium*, Lactobacillaceae, *Phascolarctobacterium*	*Butyricimonas*, Odoribacteraceae, *Odoribacter*	Nocardiaceae	*Deltaproteobacteria* Pseudomonadaceae, *Raoultella*	—	*Synergistia*, *Synergistales*, *Synergistes*

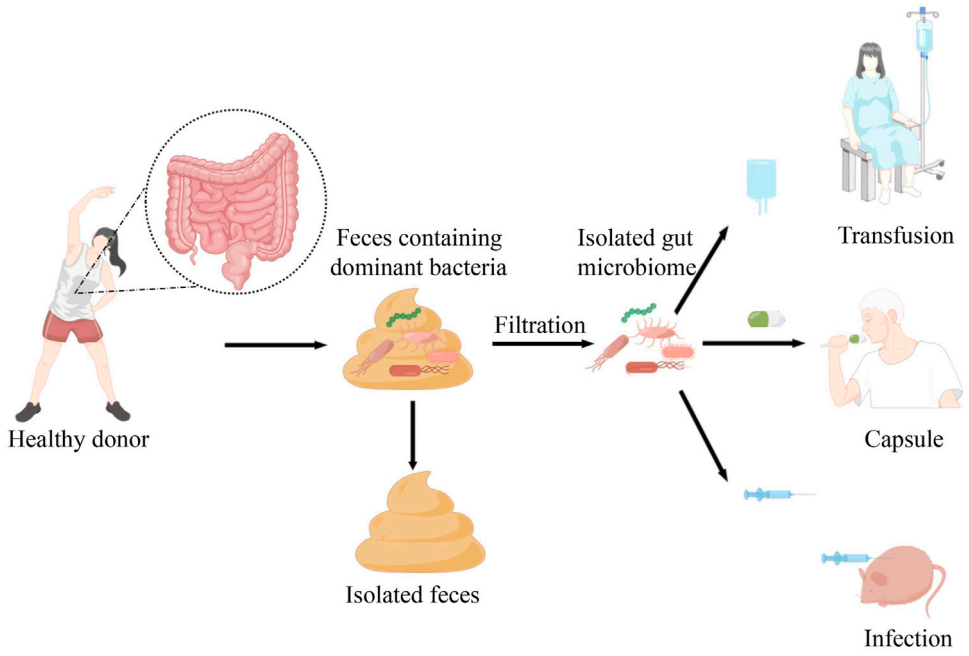

FIGURE 1
Faecal microbiota transplantation is a relatively mature approach isolating the dominant bacteria from healthy donor into the patients or mice with cancer. At present, there are three forms of faecal microbiota transplantation, including transfusion, oral administration or injection based on the manufactured bacterial fluids or capsules in order to reshape the vivo intestinal microecology.

to be neglected is that considering the heterogeneity of the relevant studies, a large number of trials are needed to explore the clinical implications of FMT (Pierrard and Seront, 2019).

4.4 Other strategies to modulate the gut microbiota in patients with cancer and treated with the ICIs

Similarly, the use of antibiotics could alter gut microbiota diversity and composition leading to dysbiosis, which may affect effectiveness of ICI. For example, patients with renal cell carcinoma (RCC), non-small-cell lung cancer (NSCLC), hepatocellular carcinoma (HCC) and so on often obtained lower OS and PFS if they were given antibiotics prior to anti-programmed cell death ligand-1 mAb monotherapy or combination therapy (Derosa et al., 2018; Schett et al., 2020; Ochi et al., 2021). Those reveal the strong relationship between the broad-spectrum ATB class and poor efficiency. Still, considering the homogeneous populations, more researches shall be carried in order to clarify these issues. Meanwhile, Clinicians shall carefully consider the use of antibiotics in cancer patients treated with ICIs (Crespin et al., 2023).

Nowadays, the use of probiotics, prebiotics and synbiotics largely enriches the interventional approaches to manipulate the microbiota. It is well known that probiotics are defined as live

microorganisms which when administered in adequate amounts confer a health benefit on the host. *Lactobacillus* and *Bifidobacterium* are the most commonly probiotics. *Lactobacillus delbrueckii* can induce cell apoptosis and inhibit the growth of human colon cancer cell (Wan et al., 2014). *Lactobacillus* spp. in colorectal cancer modulate host immunity, inhibit cell proliferation to realize anti-cancer (Wong and Yu, 2019). However, it is well known that not all *Lactobacilli* are probiotics because probiotic effects are strain-dependent. *Bifidobacterium* was demonstrated as an unexpected role for enhancing anti-tumor immunity in studies of Ayelet Sivan et al. (Sivan et al., 2015), which can improve the response of PD-1/PDL-1 inhibitors (Zhuo et al., 2019). Therefore, the advantages of probiotics are unprecedented. However, the health value of probiotics should be assessed combining multiple factors, such as clinical parameters, baseline commensal background and microbiome features considering the resistance to probiotics colonization (Zmora et al., 2018; Langella and Chatel, 2019).

Another way to enrich gut microbes that promote anti-tumor and bring benefits for consumers is through prebiotics. To date all reported prebiotics are carbohydrates. The quintessential prebiotics are inulin-type fructans, fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) (Huxley et al., 2009). They can be obtained from certain grains, fruits, nuts and vegetables, which can promote substantial alterations in the composition of fecal microbiota and commensal bacteria to produce relative metabolites (Derosa et al., 2021; Tan et al., 2022).

Synbiotics are a combination of prebiotics and probiotics that are believed to have a synergistic effect by inhibiting the growth of pathogenic bacteria and enhancing the growth of beneficial organisms. Rafter J. et al. have discovered that the combination of prebiotic inulin and the probiotics *Lactobacillus* rhamnosus GG and Bifidobacterium lactis Bb12 may change the composition of gut microbiota in patients with colonic polyps, which improved epithelial barrier function (Wong and Yu, 2019).

Efforts are required to further understand the mechanisms between the composition of intestinal microbiota and the efficacy of immunotherapy. Future research shall shed light on different animal models and prospective clinical studies to help further understand the role of intestinal microbiota. The composition of intestinal microbiota may be an essential component for cancer therapy in this fast-moving era.

5 Conclusion

Numerous studies have confirmed that gut flora plays a crucial role in the immunotherapy of cancer. Identification of specific dominant and ineffective flora can be an important basis for judging tumor prognosis and adverse events; Additionally, beneficial fecal microbiome transplantation both moderates gut flora and significantly improves the outcome. However, it is a promising therapeutic approach that still requires a very cautious and low-key approach due to the different functions of the gut microbiota in the body as a whole, and needs to be combined with clinical studies to assess the relative contribution of pre-existing bacteria that may promote transplantation versus those that against transplantation as well as the need to standardize sample configuration procedures (Lam and Goldszmid, 2021).

Targeted at immunotherapy, it is of great necessity to clarify the specific bacteria that influence the effect of immunotherapy and consider the dynamic nature of microbial communities to determine the optimal sampling point for predicting efficacy and toxicity, as well as the need to standardize sampling procedures. Furthermore, it is essential to establish a unified standard for sequencing and bioinformatics analysis to screen out prognostic biomarkers with high sensitivity and specificity.

In addition, future studies are needed to explore how basic research can be effectively translated into clinical applications, whether gut flora can be used as a potential marker for cancer, the mechanism of patient response differences for the same class of bacteria shown in different studies and how to intervene in the gut flora to overcome the challenge of patient drug resistance and so on, which may maximize immunotherapy and further reduce the incidence of immune related adverse events.

Author contributions

JM contributed to the study conception and design, and critical revision of the manuscript for important intellectual content. QW contributed to the data acquisition. XC contributed to the analysis and interpretation. JZ, ZZ, and JS contributed important guidance for this study. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

Publisher's note

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Case report: Immunotherapy plus chemotherapy and stereotactic ablative radiotherapy (ICSABR): a novel treatment combination for Epstein-Barr virus-associated lymphoepithelioma-like intrahepatic cholangiocarcinoma

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Epstein-Barr virus-associated lymphoepithelioma-like intrahepatic cholangiocarcinoma (EBVa LEL-ICC) is a rare tumor, characterized by a rich tumor immune microenvironment (TIME). While this tumor is reportedly sensitive to immunotherapy, its response has been inconsistent. This decreased sensitivity was associated with reduced TIME abundance. We report the case of a 53-year-old woman with EBVa LEL-ICC having reduced TIME abundance. The patient presented with a liver lesion, which was detected using ultrasound. Initially, the tumor was sensitive to immunotherapy and chemotherapy (IC), but resistance developed after a short interval. Subsequently, stereotactic ablative radiotherapy (SABR) was added to the patient's treatment, which now consisted of ICSABR. Successful tumor shrinkage was achieved with the combination therapy regimen. Thus, surgery and ICSABR are effective adjuncts to the first-line IC therapy in improving the survival rate of patients with EBVa LEL-ICC. The results of this study support multidisciplinary treatment as a viable treatment strategy for EBVa LEL-ICC.

KEYWORDS

Epstein-Barr virus-associated lymphoepithelioma-like intrahepatic cholangiocarcinoma, tumor immune microenvironment, immunotherapy, stereotactic ablative radiotherapy, long survival

Introduction

Lymphoepithelioma-like intrahepatic cholangiocarcinoma (LEL-ICC) is a rare tumor; it is histologically characterized by dense lymphoid infiltrates interspersed with undifferentiated epithelial cells. LEL-ICC is typically associated with the Epstein-Barr virus (EBV) infection. Hence, it is referred to as EBV-associated LEL-ICC (EBVa LEL-ICC) (Hsu et al., 1996). However, due to its low incidence rate, there is a lack of evidence about the clinicopathological characteristics and standard treatment of LEL-ICC. In previous reports, by analyzing the expression of PD-L1 in LEL-ICC and ordinary intrahepatic

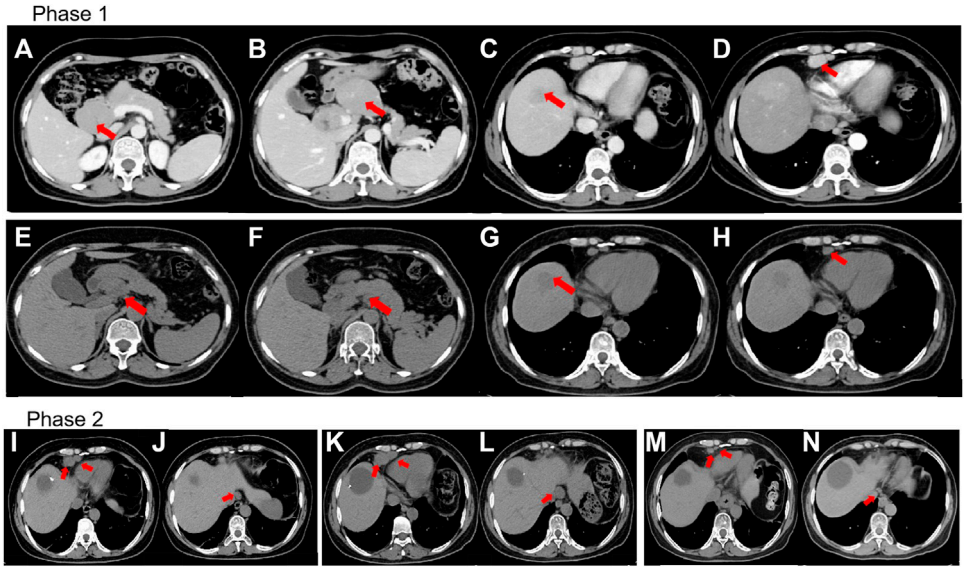

FIGURE 1
(A–D) Prior to treatment, computed tomography (CT) revealed lesions around the pancreas, liver, and lymph nodes located in the cardiophrenic angle. **(E–H)** CT revealed that lesions around the pancreas and lymph nodes reduced in size whereas the lesions proximal to the liver remained constant despite four cycles of immunotherapy and chemotherapy (IC). **(I,J)** CT revealed lymph nodes present even after mesohepatectomy and regional lymph node resection. **(K,L)** CT showed further enlargement of the lymph nodes in the cardiophrenic angle and paraesophageal region despite two cycles of IC. **(M,N)** CT revealed shrinkage of lymph nodes after stereotactic ablative radiotherapy. **(A,B)** and **(E,F)** The red arrow indicates lesions around the pancreas. **(C,G)** The red arrow indicates the lesion in the liver. **(D,H)** The red arrow indicates the lymph nodes in the cardiophrenic angle. **(I,K,M)** The red arrow indicates the lymph nodes located in the cardiophrenic angle. **(J,L,N)** The red arrow indicates the paraesophageal lymph nodes.

cholangiocarcinoma (ICC), it was found that the level of PD-L1 in LELCC was higher than that in ICC, which may indicate the sensitivity to immunotherapy.

EBV-associated cancer is significantly responsive to immunotherapy. This response has been documented in previous cases of stomach, lung, liver, and bile duct cancer (Jiang et al., 2015; Xie et al., 2020; Huang et al., 2021; Lv et al., 2022; Zhu et al., 2022). Its sensitivity to immunotherapy was related to the upregulated expression of programmed death ligand 1 (PD-L1) (Wang et al., 2022). Moreover, the efficacy of immunotherapy and chemotherapy (IC) for advanced cholangiocarcinoma with abundant $CD8^+$ T cell infiltration has also been reported; based on this, the tumor immune microenvironment (TIME) was identified as a predictive biomarker of effective immunotherapy (Zhao et al., 2021). However, case reports on the application of immunotherapy for EBVa LEL-ICC treatment are scarce. This study reports the case of EBVa LEL-ICC with reduced TIME abundance. In this study, the 53-year-old woman who presented with a liver lesion was administered with multidisciplinary treatment (MDT), which had long-term efficacy. Thus, MDT is a viable option for rare and refractory cases.

Case presentation

A 53-year-old woman presented with a liver lesion, which was detected using ultrasound. The patient had an elevated cytokeratin 19 fragment level (normal range, 30.40 U/mL), but the carcinoembryonic antigen and alpha-fetoprotein levels were normal. Abdominal contrast-enhanced computed tomography (CT) scan revealed a mass, measuring 3 × 3 cm, located in the right lateral hepatic region. Multiple metastatic lesions were also detected in the lymph nodes located in the cardiophrenic angle, hepatic hilar region, and peripancreatic region (Figures 1A–D). A core needle biopsy of the liver was performed, and the histology was consistent with LEL-ICC (Figure 2A). Immunohistochemistry (IHC) analysis revealed that the tumor was positive for PCK and EMA, but it was negative for CK7, PAX8, P63, CK8/18, hepatocyte, Arg, GPC-3, CD30, GATA-3, ER, TTF-1, ALK-1, CDX2, WT-1, and CD34. The tumor tissues were positive for EBV-encoded RNA *in situ* hybridization (Figure 2B). On next-generation sequencing (NGS), genetic aberrations were not identified due to insufficient tumor tissue. According to the eighth edition of the American Joint Committee on Cancer TNM staging system, the tumor was classified under stage IV (T4N2M1).

After multidisciplinary consultation, a combination regimen of gemcitabine (1,000 mg/m^2, d1, d8, q3w) plus cisplatin (25 mg/m^2, d1, d8, q3w) (GP) and camrelizumab (200 mg, d1, q3w) was administered on June 2021 (Figure 3). After two cycles, the CT scan showed significant regression of the peripancreatic lymph nodes. Based on the Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1), a partial response was achieved (Figures 1E, F, H). However, a slight enlargement of the liver lesion was noted during the CT scan (Figure 1G). After 4 cycles of IC, the second multidisciplinary consultation had been held, it was concluded that the IC will not lead to regression of the liver lesion; hence, surgery was suggested. On October 2021, a mesohepatectomy and regional lymph node resection were performed. The lymph node in the cardiophrenic angle was

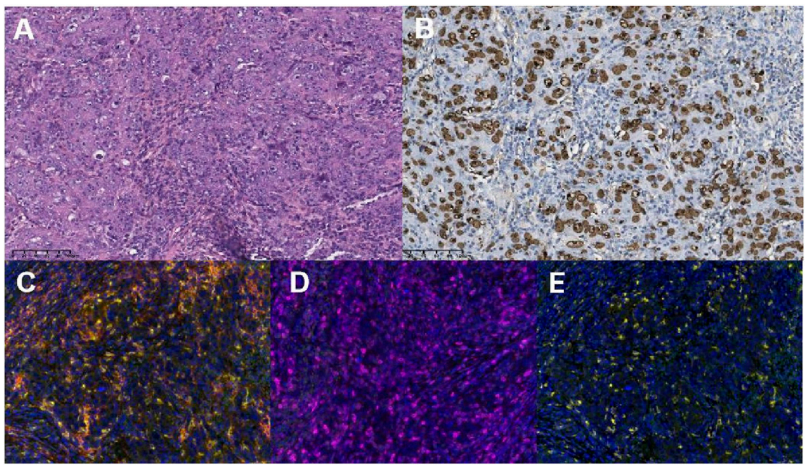

FIGURE 2
The diagnosis of lymphoepithelioma-like intrahepatic cholangiocarcinoma was pathologically verified with the expression of programmed death-ligand 1 (PD-L1), PD-1, and CD8$^+$T cells (×200 magnification). **(A)** Hematoxylin and eosin staining (×200) revealed columnar tumor cells with atypical nuclei that proliferated in a cord-like or glandular tubular pattern. The tumor cells were surrounded by collagen fibers, dense lymphocytic infiltration, and lymphoid follicles. **(B)** The brown cells in EBER-ISH×200 magnification images are the cells harboring Epstein–Barr virus (EBV) infection (EBV-encoded small RNA *in situ* hybridization, EBER-ISH). Representative examples showing immunohistochemical staining of samples that are PD-L1-positive in tumor cells (PD-1$^+$ TCs, **(C)**, PD-1-positive in tumor cells [PD-1$^+$ TCs, **(D)**], and positive for CD8$^+$T cells [CD8$^+$T, **(E)**].

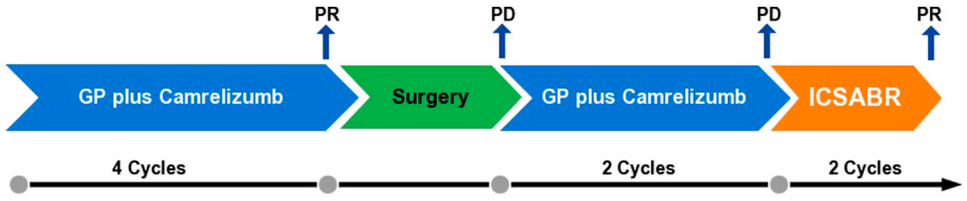

FIGURE 3
Schematic representation of the anti-tumor therapy process. First-line treatment consisted of gemcitabine (1,000 mg/m^2, d1, d8, q3w) plus cisplatin (25 mg/m^2, d1, d8, q3w) and camrelizumab (200 mg, d1, q3w). Subsequently, the surgery was performed. After the surgery, the lymph nodes enlarged; the patient was readministered with GP plus camrelizumab. After two cycles, the tumor further enlarged. The patient was administered with the stereotactic ablative radiotherapy (SABR) combined with camrelizumab and GP (ICSABR), and achieved response after two cycles.

difficult to remove due to its proximity to vascular structures (Figures 1I, J). NGS revealed that the liver tissue had a low tumor mutation burden of 3.9 Muts/Mb and a microsatellite stable status. IHC revealed that the tumor tissue had a combined positive score of 20 and a tumor proportion score of 18% (Figures 2C, D). This indicated a high level of PD-L1 expression in the tumor cells, and the CD8$^+$ T cells had infiltrated the tumor cells (Figure 2E). The TIME was examined using multiplex immunohistochemical staining and quantitative analysis (Table 1), but no gene mutations were identified.

The postoperative CT scan showed lymph node involvement in the cardiophrenic angle and paraesophageal region (Figures 1I, J). IC was considered effective in the previous treatment. Therefore, administration of IC was continued in the patient. However, the CT showed further 34% enlargement of the lymph nodes after two cycles of IC treatment, which demonstrated progressive disease (PD) (Figures 1K, L). Based on this observation, the patient was diagnosed with progressive disease. Due to the local progression, stereotactic ablative radiotherapy (SABR) for the enlarged lymph nodes with a total dose of 50 Gy was administered in five fractions. Additionally, IC was continued. After two cycles, a partial

TABLE 1 Tumor-infiltrating immune cell test results.

Test indicators (multiplex immunohistochemistry)	Test results
CD8$^+$T cells	+(2.87%)
PD-L1$^+$ cells	+(21.08%)
CD8$^+$ PD-1$^+$ T cells	+(0.97%)
CD68$^+$ macrophage cells	+(13.27%)
CD68$^+$ PD-L1$^+$ macrophage	+(12.39%)

response was achieved (Figures 1M, N). Currently, PFS reached at least 14 months.

Discussion

The application of immune checkpoint inhibitors (ICIs) in treating EBVa LEL-ICC has not been discussed in previous studies. The efficacy of immunotherapy has been documented for LEL-ICC of the lungs, breast, bladder, and liver (Iezzoni et al., 1995). Despite the lack of clinical evidence, there are biologic reasons supporting the potential efficacy of ICIs in treating EBVa LEL-ICC. Compared with the conventional cholangiocarcinoma, EBVa LEL-ICC has an increased proportion of intratumoral lymphocytes; this proportion was reportedly a predictor of the response of various cholangiocarcinoma subtypes to ICIs (Huang et al., 2021).

In this case, the patient suffered from unresectable advanced cholangiocarcinoma at the time of diagnosis. However, there was not obvious clinical symptoms or no similar family history. The ABC-02 study, published in 2010, suggested that the administration of GP in patients with locally advanced or metastatic cholangiocarcinoma was associated with a significant survival advantage without the addition of substantial toxicity. The objective response rate for this regimen was 23% (Valle et al., 2010). In the latest National Comprehensive Cancer Network guidelines, GP combined with durvalumab was recommended as the first-line treatment for advanced unresectable cholangiocarcinoma because it significantly prolonged progression-free survival and overall survival (OS) (Oh et al., 2022). A study, published in 2020 and reported by the American Society of Clinical Oncology, suggested that gemcitabine and oxaliplatin combined with camrelizumab achieved a median OS of 11.8 months (95% CI 8.3–15.4) for patients with advanced cholangiocarcinoma (Chen et al., 2020). Based on the current data on IC as the first-line treatment for cholangiocarcinoma, patients with EBVa LEL-ICC are expected to benefit from immunotherapy due to the pathological characteristics of the tumor. In this case, GP and camrelizumab were chosen.

A previous study revealed significant differences in the immune infiltrate level within the microenvironment between tumor metastasis sites (Conway et al., 2022). Metastatic liver lesions had lesser number of intratumoral lymphocytes than distant lymph node metastases, and this difference has a potential effect on immunotherapy outcomes (Conway et al., 2022). In this case, the sizes of the lymph nodes were significantly reduced, but the liver lesion exhibited minimal improvement. This was attributed to the difference in TIME between liver and lymph node metastasis. After consultation of our multidisciplinary doctors, it is thought that liver tumors were not sensitive to the IC, while tumor lesions in lymph nodes responded well. Considered that it was possible to radically treat lymph node lesions through radiotherapy in subsequent treatment, we decided to perform tumor reduction surgery on the liver lesion which also evaluating the tolerance of the patient. The TIME of the liver lesion consisted of $CD8^+$, $PD\text{-}L1^+$, and $CD8^+$ $PD\text{-}1^+$ T cells (2.87%, 21.08%, and 0.97%, respectively).

Postoperatively, enlarged lymph nodes were noted in the cardiophrenic angle and paraesophageal region. As the lymph nodes were sensitive to IC during the previous treatment, the same treatment regimen was reintroduced for two cycles. However, the expected tumor regression was not attained. Compared with a previous case of EBV-associated gastric cancer, the present case had a less abundant TIME, such as $CD8^+$ T cell density (10.59% vs. 2.87%) (Lv et al., 2022). Compared with EBVa ICC, EBVa LEL-ICC had significantly increased densities of $CD8^+$ T cells (Huang et al., 2021). The retrospective study also suggested that infiltration of the $CD8^+$ T cells significantly increased local immune activation, and this was associated with a more favorable prognosis and increased responsiveness to immunotherapy (Song et al., 2010; Huang et al., 2021). Meanwhile, in previous studies, it has been found that tumor TIME is upregulated after chemotherapy, and the induction of innate immune components, including macrophages and NK cells, drives antigen presentation. However, due to the lack of pre treatment samples from patients, it was not confirmed in our study. However, as the lesion in this case had a less abundant TIME, the patient had refractory disease with a complicated course.

Thus, MDT was performed to facilitate a more comprehensive management plan. After undergoing tumor reduction surgery, the patient underwent radiotherapy (RT) and immunotherapy for the lymph nodes. SABR is defined as a radiation dose of more than 5 Gy/fraction with a high compliance and sharp dose drop to protect the surrounding organs at risk. ISABR is a novel therapeutic option involving both SABR and immunotherapy (Weichselbaum et al., 2017). Its effectiveness has been documented in previous studies. The combination of SABR and ipilimumab, an anti-CTLA-4 monoclonal antibody, was reportedly effective in treating metastatic melanoma in 2011 (Hiniker et al., 2012; Grimaldi et al., 2014). A study in 2013 determined the absolute effect of ipilimumab combined with SABR on metastatic liver cancer (Golden et al., 2013). According to several studies, SABR stimulated the systemic immune response, thus leading to enhanced recognition of tumor cells by the immune system and neoplastic cell death (Loblaw et al., 2013; Norkus et al., 2013). In an ongoing trial COSINR, the immune signatures in tumor tissue before and after radiotherapy combined with immunotherapy were analyzed. It was found that SABR combined with ICIs increased the expression of adaptive immune and cytotoxic T cell gene programs, and improved tumor cell elimination over SABR alone. However, the study also demonstrated that SABR alone was insufficient to induce local immune augmentation. In this case, the combination of IC and SABR, ICSABR, successfully elicited a treatment response might support previous studies. This case demonstrated that ICSABR is an effective treatment option for refractory EBVa LEL-ICC. Meanwhile, the liver function and leukocyte in serum were monitored and within the normal range, which also confirmed the safety of the ICSABR. The combination treatment resulted in a marked reduction of tumor size and a more favorable long-term survival.

Conclusion

In this case, the flexible application of ICSABR in a patient with a rare and refractory EBVa LEL-ICC achieved favorable outcomes. To the best of our knowledge, this is the first report on the systematic comprehensive treatment of EBVa LEL-ICC. However, as our research was a case report, we still need randomized controlled clinical study to further verify its efficacy. With the development of

anti-tumor treatment, combining multiple therapeutic options may increase the efficacy of the treatment regimen. This case will serve as a reference for future large-scale prospective clinical studies.

Data availability statement

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

Ethics statement

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

Author contributions

DC developed the idea and revised the paper; RL and KC wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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

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