frontiers | Research Topics

Assessment and management of adverse drug reactions in oncology

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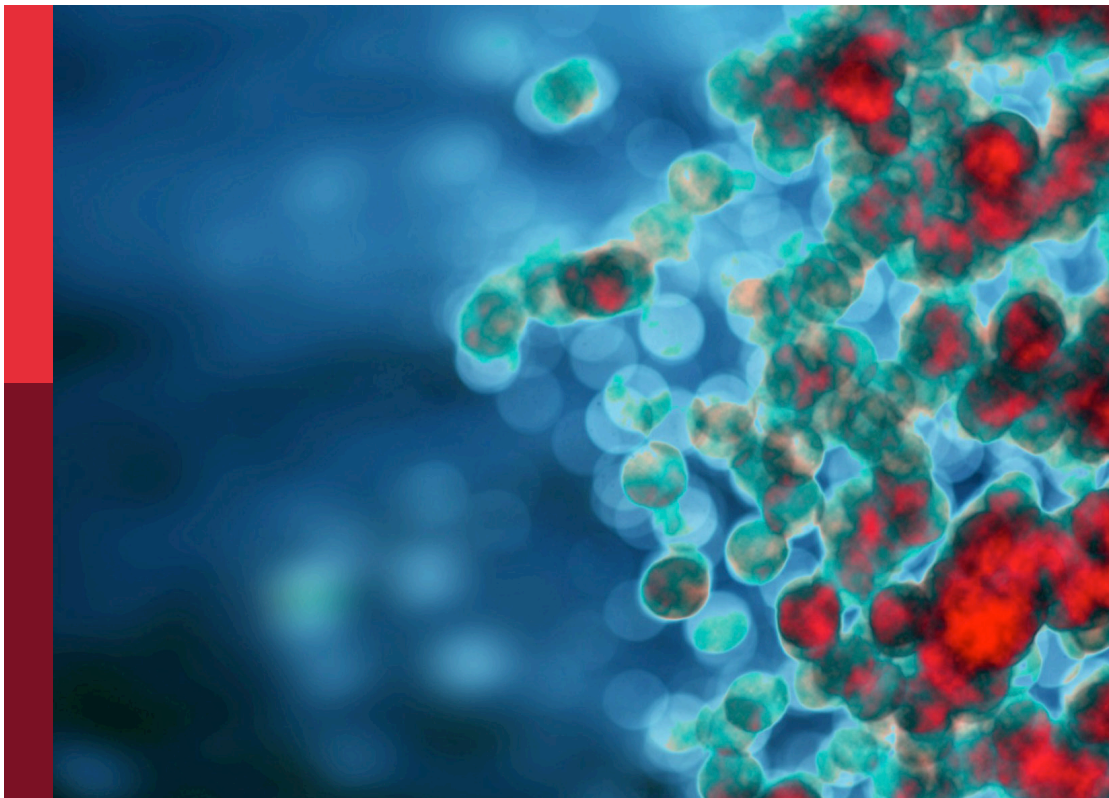
Published in
Frontiers in Immunology
Frontiers in Allergy
Frontiers in Oncology



ISSN 1664-8714
ISBN 978-2-8325-2522-7
DOI 10.3389/978-2-8325-2522-7

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Assessment and management of adverse drug reactions in oncology

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Citation

Ma, K. S.-K., Chen, C.-B., eds. (2023). *Assessment and management of adverse drug reactions in oncology*. Lausanne: Frontiers Media SA.

doi: 10.3389/978-2-8325-2522-7

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frontiers | Frontiers in Allergy

TYPE Editorial
PUBLISHED 13 July 2023
DOI 10.3389/falgy.2023.1223078

OPEN ACCESS

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RECEIVED 15 May 2023
ACCEPTED 29 May 2023
PUBLISHED 13 July 2023

CITATION
Tsai P-F and Ma KS-K (2023) Editorial: Assessment and management of adverse drug reactions in oncology.
Front. Allergy 4:1223078.
doi: 10.3389/falgy.2023.1223078

Editorial: Assessment and management of adverse drug reactions in oncology

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KEYWORDS

immune checkpoint inhibitor, immune-related adverse event, Stevens-Johnson syndrome, opportunistic infection, corticosteroid

Editorial on the Research Topic
Assessment and management of adverse drug reactions in oncology

Introduction

New drugs such as tyrosine kinase inhibitors, monoclonal antibodies, angiogenesis inhibitors, and immune checkpoint inhibitors (ICIs) have been approved by the United States Food and Drug Administration for a variety of cancer types in recent years. Despite their remarkable success in overall survival and progression-free survival in clinical trials, a substantial proportion of patients suffer from severe or fatal adverse drug reactions (1, 2). Due to the diverse manifestations of adverse events associated with anti-neoplastic agents, the assessment and management of these adverse events remain largely unknown. Among all, the assessment and management of adverse events following the use of ICIs, also known as immune-related adverse events (irAEs), is an emerging field.

Clinical presentations of irAEs

Contemporary guidelines including the Common Terminology Criteria for Adverse Events version 5.0 by the American Society of Clinical Oncology and the European Society for Medical Oncology provided the definition and grading system of organs commonly involved in irAEs, which facilitated the diagnoses of ICI-associated complications (Table 1) (3, 4). Organs commonly involved in irAEs include the skin, gastrointestinal tract, endocrine organs, and lungs; on the other hand, rare but fatal adverse events including neurotoxicity, cardiotoxicity, and pulmonary toxicity of ICIs have also been reported (3). Cutaneous toxicity is the most reported irAE. Reported manifestations of cutaneous irAEs include rashes, bullous dermatoses and severe cutaneous adverse reaction such as Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) and drug reaction with

TABLE 1 Grading system and menifestations of common immune-related adverse events.

The organ(s)	Grade 1	Grade 2	Grade 3	Grade 4
Cutaneous Toxicity	- Target lesions covering <10% BSA and not associated with skin tenderness	- Target lesions covering 10%–30% BSA and associated with skin tenderness	- Target lesions covering >30% BSA - Severe/life-threatening symptoms - Generalized exfoliative/ulcerated/bullous rash	
Colitis	- Increase of fewer than 4 stools per day	- Mucus or blood in stool - Increase of 4 to 6 stools per day	Increase of 7 stools per day; incontinence; limiting self-care ADL	- Life-threatening consequences
Hepatitis (AST, ALT increased)	<3.0 × ULN - Asymptomatic	3.0–5.0 × ULN - Asymptomatic	5.0–20.0 × ULN - Symptomatic liver dysfunction - Compensated cirrhosis - Reactivation of chronic hepatitis	>20.0 × ULN - Decompensated liver function (ascites, coagulopathy, encephalopathy, coma)
Pneumonitis	- Asymptomatic - Confined to one lobe of the lung or <25% of lung parenchyma	- Symptomatic (dyspnea, cough or chest pain) - More than one lobe of the lung or 25%–50% of lung parenchyma	- Severe symptoms (new or worsening hypoxia) - All lung lobes or >50% of lung parenchyma - Need oxygen therapy	- Life-threatening respiratory compromise (need intubation and ventilator care)
Hypophysitis	- Asymptomatic or mild symptoms	- Moderate symptoms limiting age-appropriate instrumental ADL	- Severe or medically significant limiting self-care ADL	- Life-threatening consequences (visual field impairment)
Acute kidney injury (Cr increased)	1.0–1.5 × ULN, < 1.5 × baseline	1.5–3.0 × ULN, 1.5–3.0 × baseline	3.0–6.0 × ULN, > 3.0 × baseline	>6.0 × ULN, Dialysis indicated
Myasthenia gravis	- Asymptomatic or mild symptoms	- Moderate symptoms - Limiting age-appropriate instrumental ADL	- Severe or medically significant - Limiting self-care ADL	- Life-threatening consequences (respiratory muscle involved)
Myocarditis	- Asymptomatic - Cardiac enzyme elevation or abnormal EKG	- Symptoms with moderate activity or exertion	- Severe with symptoms at rest or with minimal activity or exertion	- Life-threatening consequences (hemodynamic impairment)

BSA, body surface area; ADL, activities of daily living; AST, aspartate aminotransferase; ALT, alanine aminotrasferase; ULN, upper limit normal; Cr, creatinine; EKG, electrocardiogram.

eosinophilia and systemic symptom (DRESS). Other common manifestations of irAEs include colitis, hepatitis, nephritis, thyroiditis, hypophysitis. The incidence of cardiovascular irAEs, such as myocarditis, pericarditis and vasculitis, was generally rare yet potentially fatal (5).

Several populations are at particular high risk of irAE, which include patients receiving ICI combination therapy; for instance, more frequent and more severe cardiovascular irAEs are observed in those treated with ICI combination therapies (5). In addition, patients with pre-existing autoimmune diseases are reported to be at great risk of irAEs or complications of irAE; likewise, given the underlying mechanisms of irAEs that involve self-reactive T cells, molecular mimicry, and antigen spread, and decreased immune tolerance (3), irAE in patients on ICI may resemble autoimmune flares (6).

Infections are commonly seen in patients with irAEs. As interfering with normal immune responses that can lead to opportunistic infections, studies have proposed that ICI-induced dysregulated immunity can lead to opportunistic infections such as Mycobacterium tuberculosis (TB) infections; that said, the consensus statement by the European Society of Clinical Microbiology and Infectious Diseases suggested undetermined causation, in which it was suspected that it was immunosuppressive treatments rather than ICIs that caused the infections (7). As the clinical presentation of irAEs is versatile and the pathogenic mechanisms remained unclear, regular monitoring of end-organ function in patients undergoing anti-neoplastic treatments would benefit their overall survival and quality of life.

Diagnosis and assessment of irAEs

Drug causality assessment tools such as the algorithm of drug causality for epidermal necrolysis (ALDEN) or the Naranjo score can be used to classify the relationship between drug exposure and adverse drug reactions as 'unlikely', 'possible', 'probable', or 'definite' for severe cutaneous adverse reactions such as SJS/TEN (8, 9). However, there is currently no consensus on the diagnostic criteria of irAE due to its versatile presentation, despite the widespread prescription of ICIs in multiple cancers.

The diagnosis of irAE relies on the presence of a typical manifestation of commonly involved organs in the context of treatment with ICIs, which excludes other possible etiology. That said, while all changes in these end-organ functions as observed following the use of ICIs should suspected as a treatment-related effect, no causation could be directly inferred from the temporality; moreover, rare presentations of irAE and the diagnosis of such conditions remained challenging. As such, studies on the immunologic deviation and pathophysiology of irAE, as evidenced by biopsies and immunohistochemistry staining, are warranted to develop guidelines for the diagnosis of irAE.

Treatment modalities of irAEs

The management of irAEs depends on the severity, the involved organs, and the tumor response to ICIs. Corticosteroids are used as the primary treatment for irAE, and most patients respond well to corticosteroids (10). Recent studies showed that immune modulatory treatments for irAEs were effective and did not compromise the objective response rate and progression-free survival of cancers (11). In particular, pulse steroid therapy demonstrated a neurological improvement rate of 90% in a study with 11 immune-related encephalitis cases, it was therefore recommended for the initial treatment of immune-related encephalitis in the guideline (12) and had been used to treat ICI-associated interstitial nephritis and pneumonitis (13).

Intravenous immunoglobulin (IVIg), biological agents, such as infliximab and rituximab, and plasmapheresis, have been used to manage steroid-refractory cases of irAEs (14). Treatment response to these agents varied based on the organ involved and the immune response triggered by ICIs. With evidence on immunohistochemistry staining, the immune response mounted by ICIs against the affected organ could be revealed, which may suggest suitable immune modulators used to treat steroid-refractory cases of irAE.

The presence of irAEs and continuation of ICI treatments

Clinicians are primarily concerned about the continuation of ICI treatment. Both pembrolizumab and nivolumab have a mean elimination half-life of roughly 26 days, while the effects of programmed cell death protein 1 (PD-1) inhibitors last longer. Although some studies suggested that the presence of non-fatal irAEs may indicate better anti-neoplastic treatment response and survival outcomes (15), significant irAEs require extreme cautions when it comes to the decision of reintroducing immunotherapy, for which the management of irAEs and the judgment of whether to continue ICI treatments should be decided by a multidisciplinary tumor board that involves medical oncologists and specialists including dermatologists, ophthalmologists, cardiologists, pulmonologists, gastroenterologists, and endocrinologists.

Summary

Real-world observations on the safety of ICIs provide evidence for the epidemiology, clinical presentations, and prognosis of irAEs. The assessment and management of irAE require multidisciplinary collaborations, so as the diagnostic and treatment guidelines for irAE. This Research Topic, with valuable records of clinical experience in anti-neoplastic agent-related adverse events, reflects the global awareness of adverse drug reactions in medical oncology.

Author contributions

P-FT: draft. KS-KM: concept, draft. All authors contributed to the article and approved the submitted version.

Conflict of interest

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

Publisher's note

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frontiers | Frontiers in Immunology

TYPE Case Report
PUBLISHED 16 August 2022
DOI 10.3389/fimmu.2022.993622

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Immunology

RECEIVED 13 July 2022
ACCEPTED 28 July 2022
PUBLISHED 16 August 2022

CITATION
Hino C, Nishino K, Pham B, Jeon WJ, Nguyen M and Cao H (2022) Nivolumab plus ipilimumab induced endocrinopathy and acute interstitial nephritis in metastatic sarcomatoid renal-cell carcinoma: A case report and review of literature.
Front. Immunol. 13:993622.
doi: 10.3389/fimmu.2022.993622

Nivolumab plus ipilimumab induced endocrinopathy and acute interstitial nephritis in metastatic sarcomatoid renal-cell carcinoma: A case report and review of literature

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The prognosis of sarcomatoid renal cell carcinoma has changed dramatically with the emergence of immune checkpoint inhibitors. Notably the use of nivolumab and ipilimumab combination therapy has demonstrated promising durable therapeutic response for patients with treatment-naïve sarcomatoid renal-cell carcinoma. We present a case of 45-year-old man with a history of metastatic sarcomatoid renal cell carcinoma treated with nivolumab plus ipilimumab who developed type 1 diabetes mellitus, adrenal insufficiency, thyroiditis/hypothyroidism, and acute interstitial nephritis as a result of immunotherapy.

KEYWORDS

Sarcomatoid renal cell carcinoma (SRCC), nivolumab (PubChem SID: 178103907), ipilimumab (PubChem SID: 178103470), immune related adverse events, adrenal insufficiency, acute interstitial nephritis (AIN), hypothyroidism, diabetes mellitus

Introduction

Sarcomatoid renal-cell carcinoma (sRCC) is a rare and highly aggressive form of dedifferentiated renal carcinoma that portends an exceptionally poor prognosis (1). Approximately 60-80% of patients with sRCC present with advanced or metastatic disease at the time of diagnosis, and are known to have a poor response to traditional chemotherapy. Up until recently, the median overall survival for sRCC was approximately 6-13 months (2, 3). However, the emergence of combination immune checkpoint inhibitors (ICIs) with nivolumab (NIVO; a programmed death 1 immune

checkpoint inhibitor antibody) plus ipilimumab (IPI; a cytotoxic T-lymphocyte antigen 4 antibody) as first line therapy for advanced clear cell RCC has radically improved the survival prognosis of advanced RCC (4). More recently *post-hoc* analysis of the Phase III CHECKMATE 214 trial demonstrated promising efficacy and prolonged survival in patients with sRCC who received ipilimumab and nivolumab vs sunitinib- with a median OS of 31.2 months vs 13.6 and ORR fo 57% vs 19% (5).

Immune related adverse events (irAEs) associated with ICIs have resulted in significant morbidity through autoimmune-like toxicities (6). Although the occurrence of irAEs has been well documented in other solid tumors, the safety profile of ipilimumab and nivolumab for use in RCC has yet to be fully elucidated. Here we report a case of a 45-year-old man with metastatic sRCC who was treated with NIVO+IPI and developed several uncommon irAEs including type 1 diabetes mellitus, adrenal insufficiency, thyroiditis/hypothyroidism, and acute kidney injury likely from acute interstitial nephritis.

Case presentation

A 45 year-old male with an unremarkable past medical history and no known history of autoimmune disease, diabetes, adrenal insufficiency, or chronic kidney disease presented with severe abdominal pain, right sided lower back pain, fatigue, and night sweats was found to have a 12 x 11 x 8 cm right renal mass with tumor thrombus extending into the right renal vein and around the inferior vena cava (IVC) (Figure 1). He subsequently underwent right radical nephrectomy with retroperitoneal lymphadenectomy and partial cavectomy with bovine patch onto the cava for IVC repair. Pathology of the renal mass confirmed a diagnosis of clear cell renal carcinoma with 60% sarcomatoid differentiation and focal rhabdoid features. Sarcomatoid renal cell carcinoma was also confirmed by pathology in 3 out of 4 retrocaval lymph nodes and 1 out of 6 interaortocaval lymph nodes. In addition, tumor adherent to and excised from the IVC was positive for sarcomatoid renal cell carcinoma. Further work-up with a CT chest showed a 6 mm pulmonary nodule in the right middle lobe suspicious for metastasis. However, MRI brain showed no signs of metastasis. The patient was clinically staged T4N1M1 with G4 histologic features.

Given sarcomatoid subtype and patient's advanced disease, the patient was started on NIVO+IPI combination therapy. Within 1 week of initiation of NIVO + IPI therapy, the patient reported dark stools and was found to be anemic with Hgb of 5.1 g/dl. Further investigation revealed a 3 cm bleeding duodenal mass that was subsequently treated with coil and plug embolization of the gastroduodenal artery. A biopsy of the duodenal mass was not pursued given patient's high risk for rebleeding. Following hemodynamic stabilization, the patient was continued on NIVO + IPI.

Two months into treatment, the patient was found to have TSH of 0.074 μIU/mL with free T4 level of 2.89 pmol/L. He was subsequently referred to endocrinology for concern for immunotherapy induced thyroiditis. Approximately 2 months later, the patient's TSH increased to 63.57 μIU/mL and free T4 level decreased to 0.31 pmol/L. Due to concern for immunotherapy induced hypothyroidism, the decision was made to start levothyroxine. Follow up CT with contrast of the chest, abdomen, and pelvis showed decreased size of the duodenum and inferior vena cava masses. The 6 mm nodular mass seen in the right middle lobe on previous scan was no longer visualized.

Four months after initiation of NIVO + IPI therapy, the patient was found to have an acute kidney injury (AKI) with serum creatinine (SCr) increasing to 1.7 mg/dL from the patient's baseline SCr of 1.2 mg/dL. His blood urea nitrogen

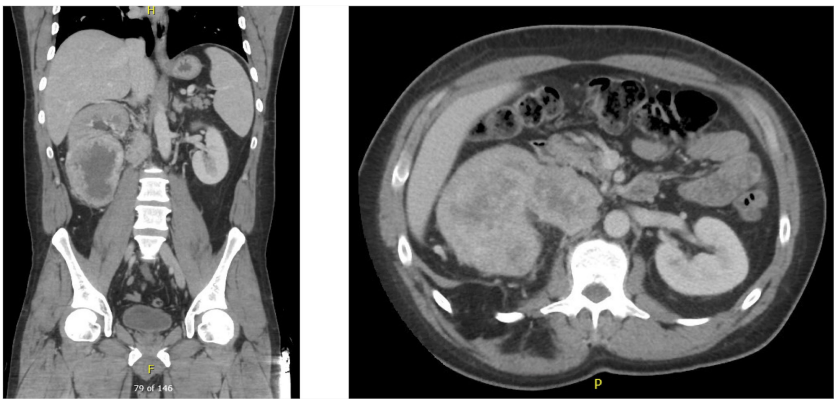

FIGURE 1
Pre-nephrectomy CT showing multiple right renal masses with tumor thrombus extending into the right renal vein and around the IVC.

(BUN) was 22 mg/dL with a BUN/creatinine ratio (BCR) of 12.9. A urinalysis at the time of AKI and 4 months prior to initiation of NIVO+IPI therapy was unremarkable for proteinuria, hematuria, pyuria, or abnormal urine sediment. Furthermore, renal ultrasonography showed no evidence for hydronephrosis or intraparenchymal disease. Microscopic evaluation for urine eosinophils showed only 0-1/hpf eosinophils/hpf. He had also been found to have to have serum eosinophil of 10.8%. Given absence of proteinuria, abnormal urine sediments, but presence of eosinophilia and eosinophiluria we had suspected mild (grade 1) immunotherapy related acute interstitial nephritis, especially in the setting of multiorgan irAE. However we could not exclude contrast induced nephropathy given recent IV contrast for imaging. Therefore, immunotherapy was held and the patient was fluid resuscitated and started on prednisone 1 mg/kg. The patient was treated with fluid resuscitation and a two week course of high-dose prednisone resulting in partial improvement in renal function with a Cr to 1.4 mg/dL. Preventative fluid resuscitation was given on a weekly basis while the patient was continued on treatment to prevent worsening of kidney function. To date, renal biopsy has not been pursued.

Eight months after starting NIVO + IPI therapy, the patient presented to hospital for one week of fatigue, worsening weakness, nausea, and blurry vision. However he denied concomitant rashes, pruritus, diarrhea or other ophthalmologic symptoms. He was found to have a blood sugar level of 667 mg/dL and hemoglobin A1c level of 7.6 on admission; thus a new diagnosis of diabetes mellitus (DM) was made. Laboratory investigation showed negative anti-glutamic acid decarboxylase antibodies, negative insulin antibodies, and low C peptide level of 0.5 ng/ml. Based on these findings, we suspect that the patient likely developed autoimmune type 1 diabetes mellitus from nivolumab therapy. He was consequently started on a daily metformin and insulin regimen.

Given our concerns for the potential side effects from immunotherapy, a decision was made to discontinue nivolumab and start sunitinib. However, the patient developed excessive fatigue, joint pain, mouth pain, thrombocytopenia and worsening Cr level to 1.9 mg/dL while on sunitinib. Sunitinib was subsequently discontinued after approximately 3 weeks of therapy, and the patient was transitioned back to nivolumab monotherapy.

Despite adequate treatment for his hypothyroidism, the patient reported progressive fatigue and memory loss for two months around the same time as he had been restarted on NIVO. Further work-up revealed a morning cortisol level of 0.3 ug/dL with an ACTH level of 3.8 pg/mL. The low ACTH and cortisol level supported the diagnosis of secondary adrenal insufficiency likely due to Nivolumab induced hypophysitis. The patient was started on treatment with hydrocortisone 25 mg BID with marked clinical improvement. The patient was resumed on immunotherapy treatment. PET scan obtained 11 months after NIVO-IPI therapy initiation showed no evidence of tumor recurrence or metastatic disease.

Discussion

Immune checkpoint molecules are crucial to the maintenance of self-tolerance and the modulation of immune mediated injury, but also play a key role in tumor immune escape (7). With the recent paradigm shift in the use of ICIs for sarcomatoid RCC, we have likewise seen both durable therapeutic responses and a concomitant change in the type of side effects observed with treatment (3). The CHECKMATE 214 trial was the first major study to document the prevalence of irAEs with NIVO + IPI combined therapy for advanced RCC (5). The reported frequency of irAEs of any grade in RCC patients treated with NIVO+IPI was found to be 81%; with dermatologic (49.8%), endocrine (33%), and gastrointestinal (29.6%) irAEs being among the most commonly reported. Treatment- related adverse events led to discontinuation in 22% of study participants, demonstrating both the severity of irAEs and the need for improved management strategies in patients with intolerable side effects (8). The case presented here highlights an exceptionally uncommon spectrum of multiorgan irAEs observed with NIVO+IPI therapy in sRCC, and the subsequent management of these multiorgan side effects (Figure 2).

ICI-induced endocrinopathies, such as adrenal insufficiency and fulminant type 1 diabetes mellitus are extremely rare and potentially life-threatening conditions that were observed in the patient discussed above (9, 10). The reported incidence of adrenal insufficiency with NIVO+IPI therapy ranges between 0.7- 29%, however the known incidence in RCC is reported between 4.7-10.2% (8–13). This discrepancy is most likely due to the limited sample size of current studies and infrequent recognition of adrenal insufficiency in these patients. In our case, the vague presenting symptoms of progressive fatigue and low energy prompted early evaluation and treatment for adrenal insufficiency. While use of high dose corticosteroids may temporarily alleviate symptoms, ICI-induced adrenal insufficiency is rarely reversible and often requires long term glucocorticoid replacement (12).

The development of type I diabetes mellitus (T1DM) is an even more rare manifestation of ICIs, with an estimated incidence between 0.2 and 1.4% (12, 14–18). Previous meta-analysis has shown that the onset of type 1 diabetes mellitus often occurs within the first 3 months of starting therapy. Remarkably only half of previously reported cases were found to have detectable type 1 diabetes-associated antibodies at presentation. By comparison ~90% of people with prototypical childhood-onset type 1 diabetes have one or more antibodies at diagnosis (17). Our patient was not found with anti-GAD or anti-islet antibodies at the time of presentation, however his low

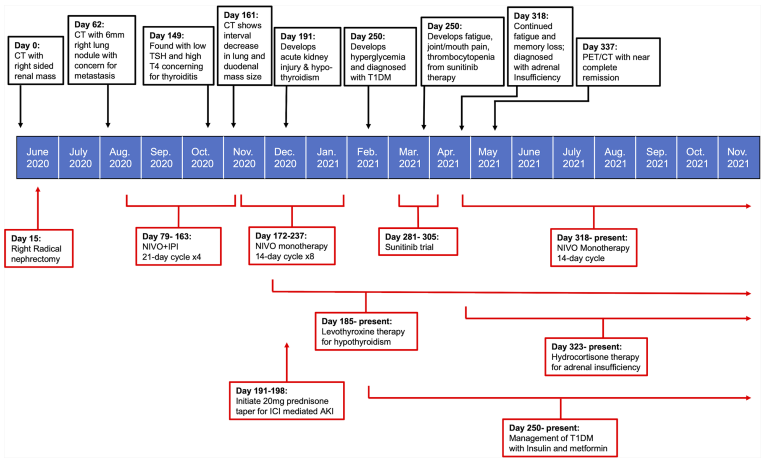

FIGURE 2
Timeline summarizing patient's treatment course in relation to onset and management of irAEs.

C-peptide, insulin deficiency, and unexplained hyperglycemia prompted treatment with exogenous insulin therapy for T1DM. The management of diabetes is challenging in the present case, especially given that use of steroids was indicated for management of the patient's other presenting irAEs, but also appears to be ineffective in reversing beta cell dysfunction and may even worsen insulin resistance (14).

Thyroid dysfunction is the most frequently cited ICI-endocrinopathy. It can occur within 3 weeks to 10 months following ICI therapy, with a frequency between 5.2- 28% with combination ICI therapy. While Anti-PD1/PDL-1 agents are predominantly associated with thyroid irAEs, recent studies suggest that patients receiving combination ICI therapies are at greater risk for thyroid dysfunction (9, 10). The patient described above developed thyroiditis which eventually progressed to overt hypothyroidism. In most cases, hypothyroidism remains permanent and requires long-term levothyroxine replacement as seen in this patient (10).

The incidence rate of ICI-induced acute interstitial nephritis in metastatic renal cell carcinoma is estimated to be 1.7% (19, 20). Given the acute rise in creatinine, eosinophilia, eosinophiluria we suspect that our patient developed ICI-induced acute interstitial nephritis. However no kidney biopsy was obtained to definitively confirm the diagnosis. The difficulty in diagnosing AIN in the present case was complicated by recent contrast use and development of uncontrolled DM, however recovery of renal function following ICI discontinuation and steroid administration points to the likely diagnosis of AIN.

While guidelines have been established for the management of common irAEs, the current case demonstrates 1) the need to establish strategies for managing multiorgan irAEs and 2) the importance of monitoring for uncommon but potentially life-threatening manifestations. Here we show that despite a promising response to therapy, the management of multiple concurrent irAEs may complicate and potentially interfere with the ability to continue ICIs in the future. This raises a particularly relevant and interesting dilemma that has not yet fully been addressed in the literature: Should patients with good initial response to ICIs continue/restart therapy in the setting of moderate-severe irAEs?

Accumulating evidence suggests that the increased occurrence of irAEs is associated with durable therapeutic responses with ICIs (20–29). This association has also been independently observed in mRCC (30–35). The current case adds support to the literature, suggesting that patients with severe multiorgan irAEs may have improved response to ICI therapy. Based on these observations, it is reasonable to hypothesize that irAEs are a byproduct of a hyper-responsive immune state that simultaneously promotes anti-tumor immunity and autoimmunity.

Previous studies have observed that patients frequently developed eosinophilia prior to the onset of ICI induced adrenal insufficiency and that eosinophilia may be an independent predictor for a favorable response to therapy (13, 36–40). However it should be noted that eosinophilia can also be an early sign of adrenal insufficiency independent of ICI therapy (41). ICI-induced acute interstitial nephritis, which is also associated with eosinophilia, has additionally been implicated in predicting a favorable response to ICIs in RCC (19). The current case corroborates these findings, and may point towards a shared uncharacterized mechanism between irEAs and effective ICI response.

The similarity between irAEs observed in our patient and the polyendocrinopathy characterized in IPEX syndrome may allude to the critical role of regulatory T cells (Tregs) in mediating both the clinical efficacy and irAEs associated with ICI therapy. Treg dysfunction through mutation in its master transcription factor, FOXP3, drives the immune dysregulation

observed in IPEX (immunodysregulation polyendocrinopathy enteropathy X-linked) syndrome (42). It would thus seem reasonable that a blockade of immune checkpoints expressed on intratumoral Tregs such as PD-1 and CTLA-4 could promote an IPEX-like syndrome, as observed in the present case.

Current guidelines recommend permanent discontinuation of ICIs that induce severe grade 4 irAEs (6, 43). However, recent studies have suggested that it may be conceivable to rechallenge patients with the same ICI after resolution of symptoms (44). In support of this, a recent large retrospective cohort study observed only a 28.8% irAE recurrence rate following rechallenge with the same inciting ICI (45). In the present case, we did not observe additional irAE following rechallenge with NIVO monotherapy. This strengthens the possibility that ICI rechallenge may be beneficial in sRCC patients who respond well to initial therapy.

Conclusions

In conclusion, ICI- associated endocrinopathies and acute interstitial nephritis are rare irAEs, but are important to recognize with NIVO+IPI therapy in sRCC. Clinicians should have a high index of suspicion for these uncommon manifestations, which should prompt urgent evaluation, discontinuation of therapy, and initiation of appropriate irAE therapy when indicated. Future studies should determine the most appropriate management strategy for patients with sRCC who develop severe irAEs despite good response to ICI 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 author.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

CH, KN, BP, WJ wrote and edited the manuscript. MN and HC contributed to the diagnosis and treatment of case, and revised the paper. All authors read, approved the submitted version, and agreed to be accountable for all aspects of the research in ensuring the accuracy of this study. All authors have given consent to the publication of this manuscript.

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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frontiers | Frontiers in Immunology

TYPE Case Report
PUBLISHED 22 August 2022
DOI 10.3389/fimmu.2022.972302

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Immunology

RECEIVED 18 June 2022
ACCEPTED 21 July 2022
PUBLISHED 22 August 2022

CITATION
Pang L, Chen Z, Xu D and Cheng W (2022) Case Report: Case report: *Mycobacterium neoaurum* infection during ICI therapy in a hepatocellular carcinoma patient with psoriasis. *Front. Immunol.* 13:972302. doi: 10.3389/fimmu.2022.972302

Case report: *Mycobacterium neoaurum* infection during ICI therapy in a hepatocellular carcinoma patient with psoriasis

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We report here a patient with advanced hepatocellular carcinoma (HCC) and psoriasis treated with immune checkpoint inhibitor (ICI) therapy who experienced tumor partial response and psoriatic exacerbation. Meanwhile, the patient contracted *mycobacterium neoaurum* during the treatment period, while it was an opportunistic infection and mainly happened in immunosuppressed patients. We discussed the possibility that this infection was an ICI-associated infection independent of immunosuppression due to dysregulated immunity, which was the result of the effects of immunotherapy and autoimmune disease (AID), and the characteristics and treatment of *M. neoaurum*, which was rarely reported in China. This case highlights the fact that some infections can be precipitated by ICIs in the absence of immunosuppressive treatment, especially the patients with AID.

KEYWORDS

immune checkpoint inhibitor (ICI), *mycobacterium neoaurum* infection, psoriasis, hepatocellular carcinoma, case report

Introduction

Immune checkpoint inhibitors (ICIs), including anti-cytotoxic T lymphocyte antigen 4 (CTLA-4), anti-programmed cell death 1 (PD-1), and anti-programmed cell death 1 ligand 1 (PD-L1) antibodies, are novel agents approved for the treatment of late-stage malignancies in recent years, including advanced hepatocellular carcinoma (HCC). Sorafenib has been the only systemic treatment option for patients with advanced HCC in the past (1). At present, combination treatment with an ICI and an anti-vascular endothelial growth factor (anti-VEGF) agent has been an effective strategy (2). However, the majority of patients suffer from immune-related adverse events (irAEs), with an incidence between 54% and 76%, according to a metanalysis of trial data (3), especially in

patients with autoimmune disease (AID) (4), so irAEs partly restrict their use. Moreover, the precise mechanism underlying irAEs is unknown, maybe due to dysregulated immunity (5).

Here, we report the first case of *Mycobacterium neoaurum* infection in China mainland, in an adult man with advanced HCC and psoriasis under ICI immunotherapy. We believe that dysregulated immunity caused by immunotherapy and AID counterintuitively favors the pathogen.

Patient description

A 53-year-old Chinese man has more than 20 years of history of chronic hepatitis B virus (HBV) infection and psoriasis without standardized examination and treatment in the past. Other than that, no other underlying diseases. In January 2021, he was admitted with psoriasis. At that time, he had minor psoriatic lesions on his trunk and extremities. Chest computed tomography (CT) happened to find multiple low-density lesions in the liver. Subsequently, the liver magnetic resonance imaging (MRI) showed multiple substantial lesions, which were suspected neoplastic lesions. It was identified as HCC by testing hepatic puncture biopsy. Hilar hepatic and retroperitoneal lymph nodes metastasis were observed in positron emission tomography/computed tomography (PET/CT). Alpha-fetoprotein was significantly elevated (>1200 μg/L). The patient was Child-Pugh A with elevated HBV DNA load with an Eastern Cooperative Oncology Group (ECOG) performance status of 0. And immunohistochemistry of tumor tissue showed AFP (±), CD34 (vascular +), CK19 (–), CK20 (–), CK7 (scattered +), CK-P (+), GPC (-), Hepatocyte (+), Ki-67 (LI about 10%), Vimentin (-). CNLC IIIb HCC was diagnosed in February 2021.

He was started on anti-hepatitis B virus therapy. Treatment for HCC did not begin with lenvatinib until his HBV DNA load dropped to normal levels. Two months later, the treatment regimen was adjusted to lenvatinib in combination with camrelizumab. The assessment of efficacy is partial remission (PR) after completing the combination for three cycles.

During the 6th course of camrelizumab, MRI suggested liver tumor progression. At that time, his psoriasis progressed with obvious pain and skin lesions expanding on both hands, legs, and trunk (Figure 1). We treated with topical corticosteroids and body balm and withdraw the next immunotherapy. Subsequently, a minor clinical improvement of the psoriatic lesions was noted. Finally, a skin biopsy confirmed the diagnosis of psoriasis (Figure 2).

After the 6th course of camrelizumab, the patient began to experience intermittent fatigue and chills without fever and blood tests were normal. He presented to the hospital for the 8th course on schedule. His laboratory results showed normal WBC count and differential at the time of admission, except for slightly high monocytes. But suddenly, he had a fever of 39°C and a loss of appetite. We did a blood culture, and his symptom gradually improved without any treatment. Seven days later, the blood culture grew *M. neoaurum* (Figure 3). Given the limitations like diagnostic qualifications and laboratory biosafety, the patient went to another two hospitals, and the same results were reported in multiple blood cultures. Antibiotic susceptibility testing was performed on this isolate, suggesting that it was resistant to all commonly used anti-

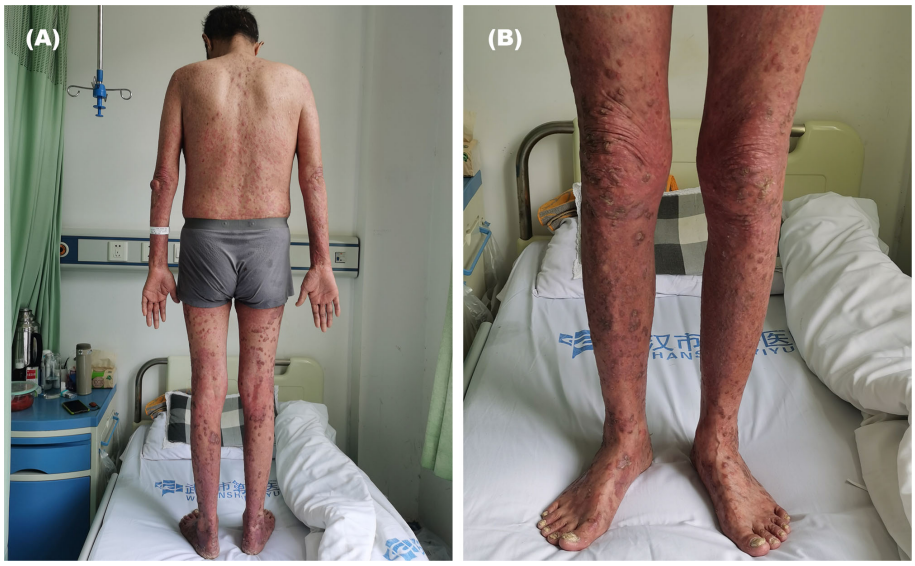

FIGURE 1
Psoriasis exacerbated during the 6th course of anti-programmed cell death 1 (PD-1) (camrelizumab), which characterized by skin lesions expanding on both hands, legs, and trunk. (**A**, back of the whole body), (**B**, front of the legs).

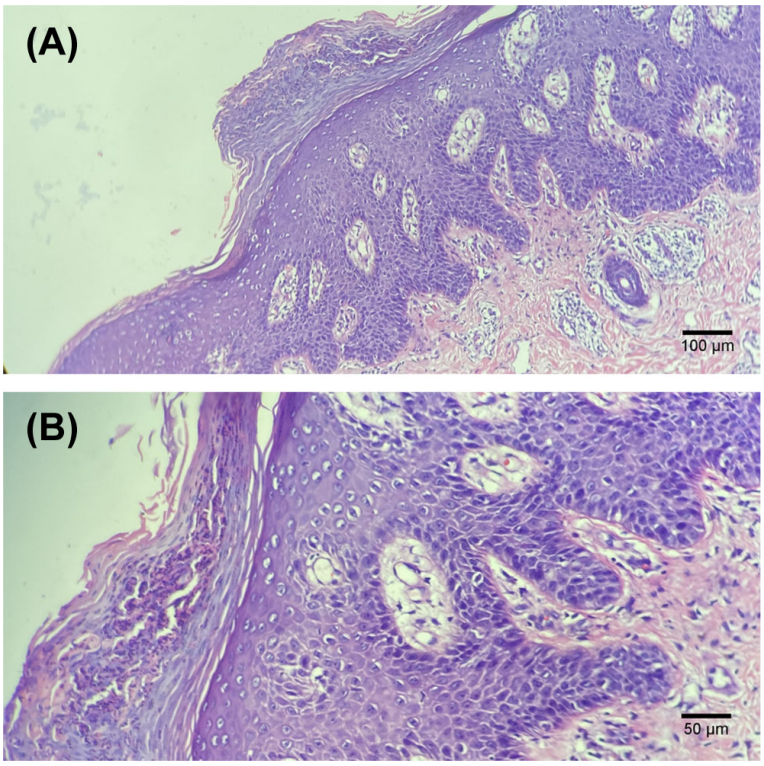

FIGURE 2
Histological examination of the skin biopsy showing typical features of psoriasis, i.e. thickened epidermis with hyperkeratosis and regular acanthosis, neutrophils trapped in the stratum corneum (Munro micro-abscesses), considerable oedema of the dermis and slight lymphocyte infiltrate around dermal capillaries. Hematoxylin and eosin staining; (**A**, 100× images), (**B**, 200×images).

mycobacterial drugs including ceftriaxone, imipenem, amikacin, ciprofloxacin, levofloxacin, sulfamethoxazole-trimethoprim, and ertapenem. Considering his skin damage and contact with aquatic products, we thought that *M. neoaurum* bacteremia may be from a cutaneous infection. The optimal therapy for infections caused by *M. neoaurum* has not been established, and the single drug is also exploratory, providing individualized treatment experience for future treatment and reducing drug resistance. Furthermore, the patient strongly requested to keep the infusion port when we informed the patient that may be from the implantable venous access port (PORT). Thus, the patient received oral contezolid and suspended camrelizumab during treatment for *M. neoaurum* infection. Ten days later, the blood culture was negative. Two cycles of contezolid were continued due to bacteremia.

Unfortunately, his HCC significantly progressed 2 months after stopping immunotherapy, and distant osseous metastases were found. Thus, a single fraction of palliative radiotherapy was delivered to bone lesions (40 Gy) to alleviate his pain. Systemic therapy with camrelizumab continued. Four weeks later, he fevered again, and two sets of blood culture specimens, from the PORT, implanted for 11 months, and a peripheral vein respectively, were collected. The blood culture collected from the POPT grew *M. neoaurum*, and his catheter was reluctantly and immediately removed. He received contezolid for 2 weeks again with the resolution of bacteremia.

Discussion

To our knowledge, this is the first case of *M. neoaurum* bacteremia during ICI therapy. ICIs theoretically do not seem to directly increase the risk of infection (6) but rather reactivate the cytotoxic T cells that were suppressed by the cancer cells to attack the tumor cells and enhance the immune system (7, 8). It was believed that concomitant use of immunosuppressants including systemic corticosteroids with ICIs treatment increased infection risk (9). However, research has found that no significant difference in the use of immunosuppressive agents between patients with and without infections (10, 11). Morelli et al. suggested that the role of a dysregulated immunity whose mounted response paradoxically favors the pathogen (9). We think that it may be immunotherapy infection due to dysregulated immunity, especially when the patient with psoriasis, which can exacerbate immune disorders.

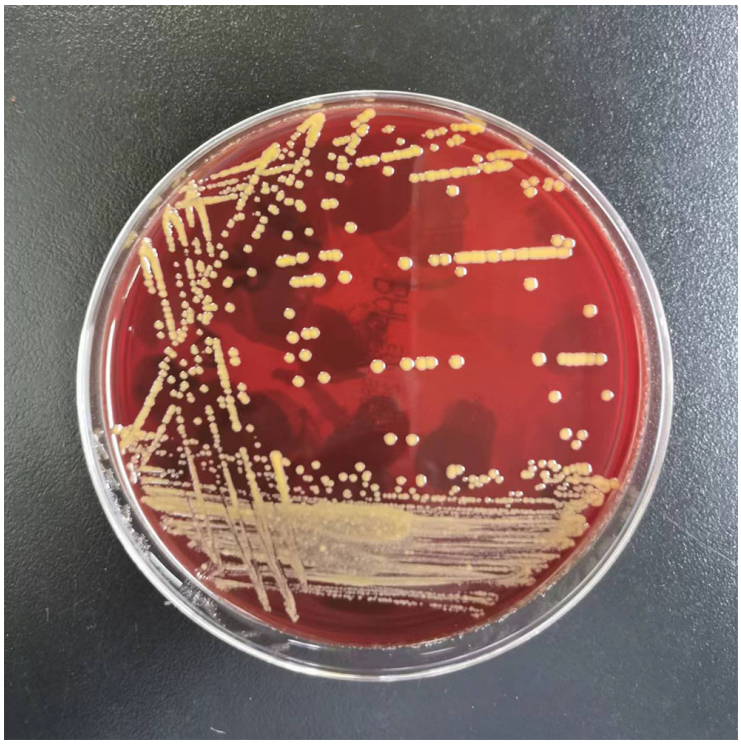

FIGURE 3
Mycobacterium neoaurum growth on blood plate. Note the smooth daffodil yellow appearance of colonies.

M. neoaurum, a member of nontuberculous mycobacteria (NTM), found in the environment, particularly in water and soil, was initially isolated from soil by Tsukamura in 1972 (12) and then described as a human pathogen for the first time in 1988 (13). So far there are only 28 cases from the literature to define the management of this rare infection. *M. neoaurum* infection mainly causes catheter-associated bloodstream infection (CABI) and develops in immune-compromised populations (14, 15). Our patient was diagnosed with CABI without immunocompromised, but we believed that ICIs seem to directly increase the risk of infection and the hyperinflammatory dysregulated immunity associated with ICIs drives this infection. Furthermore, in this case, the drug sensitivity test suggested all commonly used anti-mycobacterial drugs resistance, and we thought it was CABI after ruling out the source of skin infection, we removed the catheter and applied contezolid, a new generation of oxazolidinone antibiotics developed independently in China, with antibacterial activity similar to linezolid (16). In contrast, contezolid has fewer adverse effects on myelosuppression and better clinical application. Considering that the optimal therapy for infections caused by *M. neoaurum* has not been established and the single drug is also exploratory, we try to provide an individualized treatment experience for future treatment and reduce drug resistance in this case.

Before being diagnosed with advanced HCC, the patient had psoriasis for many years, without systemic therapy. However, lesions exacerbated during the 6th course of camrelizumab. The safety and efficacy of ICIs in patients with cancer and pre-existing AID are still a key issues to be addressed. Several studies have suggested that patients with AID may easier to occur exacerbation of AID and irAEs (4, 14, 15, 17), while in 56 patients with non-small cell lung cancer (NSCLC) with AID treated with a PD-(L)1 inhibitor, the incidence of irAEs was similar to reported rates in clinical trials where patients with AID were excluded (12). In addition, based on the current evidence from retrospective studies, most irAEs were manageable with corticosteroids in these patients (4). Thus, there seems no reason to exclude these patients from cancer immunotherapy even though patients with AID may be at higher risk of developing irAEs or have more severe irAEs. A systematic review suggested that half of the patients with AID had exacerbation of pre-existing AID, generally had the same manifestations as those occurring before ICI therapy, and the most commonly reported AID was psoriatic arthritis and/or psoriasis (22.8%) (4). Close monitoring and timely treatment can deal with most problems (13). Certainly, future prospective studies are needed to provide more favorable evidence.

Our patient, with advanced HCC and psoriasis under ICI immunotherapy, suffered *M. neoaurum* infection. Although there was a lack of more indicators to assess the immune status of the body, we think that the hyperinflammatory

dysregulated immunity associated with ICI drove this special pathogen infection, which supported the hypothesis that immune dysregulation under ICI predisposes patients to opportunistic infections including *M. neoaurum*. Clinical vigilance and early diagnosis are important to prevent severe infection and continuity of anti-tumor treatment. On the other hand, there is still a debate about whether patients with AID can receive ICIs treatment. IrAEs in patients with AID, who are receiving ICIs, can often be managed without discontinuing therapy, although some events may be severe and fatal (4, 18, 19). Moreover, close monitoring irAEs, including the occurrence of irAEs and the flares of the AID, and a multidisciplinary approach should be realized.

Patient perspective

During the whole process, the patient and his wife were informed about treatment options, risk, and possibility of relapse. They were aware of the complexity of his unusual case, and the patient provided written informed consent for the publication of his case.

Data availability statement

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

Ethics statement

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

Author contributions

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

Conflict of interest

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

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frontiers | Frontiers in Immunology

TYPE Systematic Review
PUBLISHED 26 August 2022
DOI 10.3389/fimmu.2022.1002034

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Immunology

RECEIVED 24 July 2022
ACCEPTED 09 August 2022
PUBLISHED 26 August 2022

CITATION
Zhao F, Yu R, Chen S, Zhao S, Sun L, Xu Z, Zhang Y, Dai S, Zhang G and Shu Q (2022) Global research trends on precision cancer medicine-related rashes (2008-2021): A bibliographic study. *Front. Immunol.* 13:1002034. doi: 10.3389/fimmu.2022.1002034

Global research trends on precision cancer medicine-related rashes (2008-2021): A bibliographic study

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Background: Precision cancer medicine-related rashes are a kind of skin and mucous lesions caused by precision therapy. More and more evidences indicated that such events should not be ignored in the course of anti-tumor therapy. Since cancer treatment entered the "Precision Era", there has been a rapid increase in this field. However, there was few bibliometric studies to provide an overall review of this field. This study aims to evaluate the literature output and trends in researches on precision cancer medicine-related rashes from a global perspective.

Methods: Collected publications on precision cancer medicine-related rashes from the Web of Science Core Collection database, which were limited to articles and reviews in English. Microsoft Excel, VOS viewer and CiteSpace V were used for quantitative and visual analysis.

Results: A total of 1,229 papers were identified. From 2008 to 2021, annual publications increased year by year. The United States published the most papers in this field (44.9%) and ranking first in citation frequency (19,854 times) and H-index (69). The University of Texas system ranks first with 98 papers published. Lacouture M.E and Robert C were the principal investigators. Cancers has the largest number of articles published, with 70 articles. In recent years, there have been research hotspots related to immunotherapy, including ipilimumab, immunotherapy, tumor microenvironment, association, checkpoint inhibitor, and cutaneous adverse event.

Conclusion: Precision cancer medicine-related rashes are a hot research topic in oncology. The number of relevant publications will increase dramatically. "Checkpoint inhibitors", "skin adverse events", "associations" and "tumor microenvironment" may become research hotspots in the future.

KEYWORDS

precision cancer medicine, targeted therapy, checkpoint inhibitors, rash, bibliometric analysis

Introduction

The field of precision cancer medicine is constantly broadening with the biotechnological breakthroughs. It brings safer and more novel therapies to patients, such as gene-targeted therapy, immune-targeted approaches, etc. (1). Clinical trials have shown that patients receiving targeted therapy matched to their molecular changes had better response, time to treatment failure and survival than patients receiving unmatched therapy, and progression-free survival could be improved by approximately 30% (2). Immunotherapy, including checkpoint blockade, personalized vaccines, etc, demonstrated a survival benefit versus chemotherapy, exhibiting a five-fold increase in 5 years' overall survival (OS) rate (13.4% vs 2.6%) (3, 4).

However, these precision therapies also generate various toxicities, mainly involving the skin, gut, liver, lung, and endocrine glands, but can potentially affect any tissue (5, 6). Rashes are one of the most common adverse reactions, which often profoundly reduces patients' quality of life (7–9), affects the treatment outcomes. It has become a major challenge in accelerating the implementation of precision medicine. Over the past few years, the accumulating evidence has shown that the occurrence of rashes may herald better efficacy of precision therapy. The pathogenesis, diagnosis, prevention and treatment of precision cancer medicine-related rashes have received considerable attention, and many scholars have published relevant research articles. To our knowledge, few systematic investigations have been conducted on the scientific output and current status of research on precision cancer medicine-related rashes from a global perspective.

In this study, we performed a bibliometric analysis to systematically evaluate studies of precision cancer medicine-related rashes. We combine statistical methods with data visualization to analyze the bibliography of relevant literature to identify global research trends and hotspots in this field.

Materials and methods

Data retrieval and literature screening

In this study, the Science Citation Index (SCI) Expanded of Web of Science Core Collection (WoSCC) database was chosen as the data source. Searches were conducted using the following search strategy: ("Immunotherapies" or "Molecular Targeted Therapies" or "Targeted Therapy" or "Molecular Targeted" or "Targeted Molecular Therapy" or "Molecular Therapy" or "Targeted" or "Targeted Molecular") and ("Skin Rash" or "Rash" or "Skin" or "Exanthem"). The time is from the inception of the database to March 28, 2022. Language was restricted to English. For manuscript types, we selected original articles and reviews, and excluded all other source types to ensure quality research (Figure 1).

Data extraction and analysis

The data were extracted independently by two authors, including annual study, countries, institutions, authors, journals, citations, and keywords. We used Microsoft Excel 2021 for quantitative analysis to calculate the total number of annual publications and the average citations of per publication, the number of annual publications and cumulative number over the years in countries, and the cumulative number of papers published by institutions, authors and journals. As evaluation metrics for publications, we mainly used Impact Factor (IF) and category data published by Journal Citation Reports (JCR) in 2021 to assess the quality of scientific information. The H-index (10) was also used to assess researchers' scientific output and academic standing, as well as the productivity and influence of countries, institutions, and journals. The H-index means that if a researcher's H-index is h, then the researcher has published at least h papers, and each paper has been cited at least h times.

For the visualized analyses, VOS viewer (version5.8 R3) was used for co-authorship and co-citation analyses of countries, institutions, authors, journals, and co-occurrence analyses of keywords. CiteSpace V (version5.8 R3) was used to create the dual map overlay of journals, and generate a timeline view of keywords. Each node in the graph represents different parameters including countries, institutions, keywords, etc. The weighting of parameters determined the size of the nodes, such as the number of publications, the number of citations, or the frequency of occurrence. The higher the weight, the larger the node. Nodes and lines are colored by the cluster they belong to. Lines between nodes represent links. The Total Link Strength

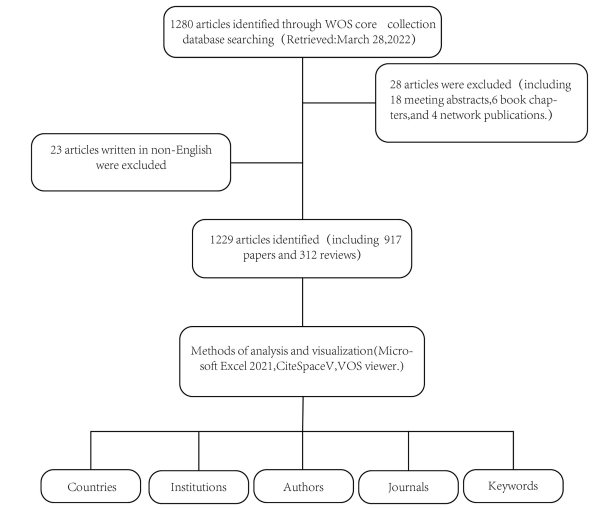

FIGURE 1
Flowchart for the selection of publications included in this study.

(TLS) indicates the total co-authorship and co-citation link strength between countries, institutions and authors.

Research ethics

Ethical approval was not required in our study, as the data used in this study were downloaded from public databases and did not involve any new studies in humans or animals.

Results

Publication outputs and citation trend

A total of 1,229 articles on precision cancer medicine-related rashes including 917 papers and 312 reviews were identified from WOSCC database on March 28,2022. The number of publications from 2008 to 2021 has been increasing, especially in the past two years significantly (Figure 2). According to the search results, the sum number of citations was 31,569, and the average number of citations was 25.69. In addition, the total citation frequency of the included articles was 35,101 times, and the average citation frequency of the articles was 28.56 times. H-index of the academic field was 83 during this time period, indicating that the academic output in this field had research value and prospect.

Distribution of countries

Table 1 lists the top 10 countries with the most articles that associated with precision cancer medicine-related rashes, and Figure 3A shows changing trend of the number of relevant publications in these countries from 2008 to 2021. The United States had the largest number of publications, accounting for 44.9% of the total number of articles (552/1,229), followed by China (12.4%, 152/1,229) and Italy (9.4%, 116/1,229). The publications from the United States were cited the most (19,854 times) and had the highest H-index (69). The total number of articles overlapped because of cooperation between countries. Figure 3B shows the literature citation relationship between countries. The largest TLS of country was United States (TLS=322), followed by German (TLS=167) and France (TLS=136).

Distribution of institutions

A total of 2,057 institutions published articles in precision cancer medicine-related rashes. The collaboration between institutions had allowed more agencies to participate in this area. The top 10 institutions ranked by the number of articles are showed in Table 2. The vast majority of institutions were from the United States. University of Texas system contributed the most publications, followed by UTMD Anderson Cancer Center and Harvard University. University of Texas system had the highest H-index, and Unicancer from France had the most average citation per article.

Figures 4A, B show the institutions' collaboration and citation network visualization map generated by VOS viewer (version5.8 R3). As shown in the Figure 4A, the map had 150 items and 967 links. The 150 items were grouped into 11clusters based on color. It meant that the institutions in the same cluster were closed related. The network map of citation analysis in Figure 4B presented 144 items and 1,048 links. The top

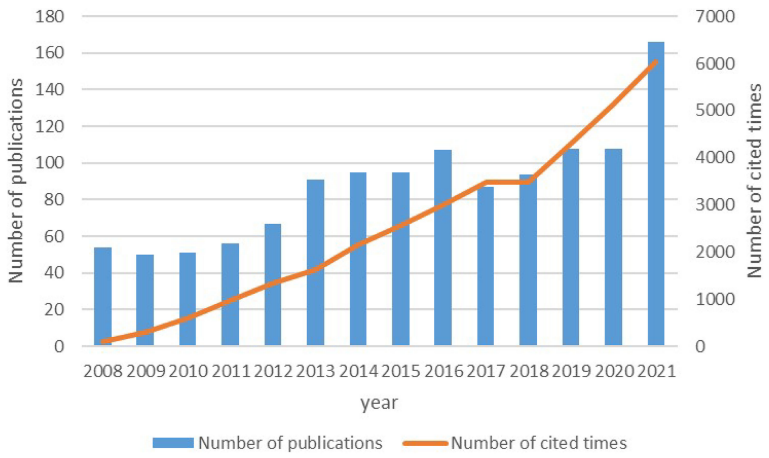

FIGURE 2
Trend of the number of articles published annually and the total amount of citations of annual articles.

TABLE 1 The top 10 productive countries with publications.

Rank	Countries	Article count	Percentage (n/1229)	H-index	TLS	Total citations	Average citation per article
1	United States	552	44.90%	69	322	19,854	35.97
2	China	152	12.40%	26	80	2,930	19.28
3	Italy	116	9.40%	31	130	4,578	39.47
4	Germany	113	9.20%	32	167	4,242	37.54
5	France	90	7.30%	34	136	5,954	66.16
6	England	82	6.70%	31	131	4,390	53.54
7	Japan	63	5.10%	23	53	2,413	38.2
8	Canada	59	4.80%	28	81	2,160	36.61
9	Netherlands	52	4.20%	26	105	2,314	44.5
10	Spain	51	4.10%	23	109	2,000	39.22

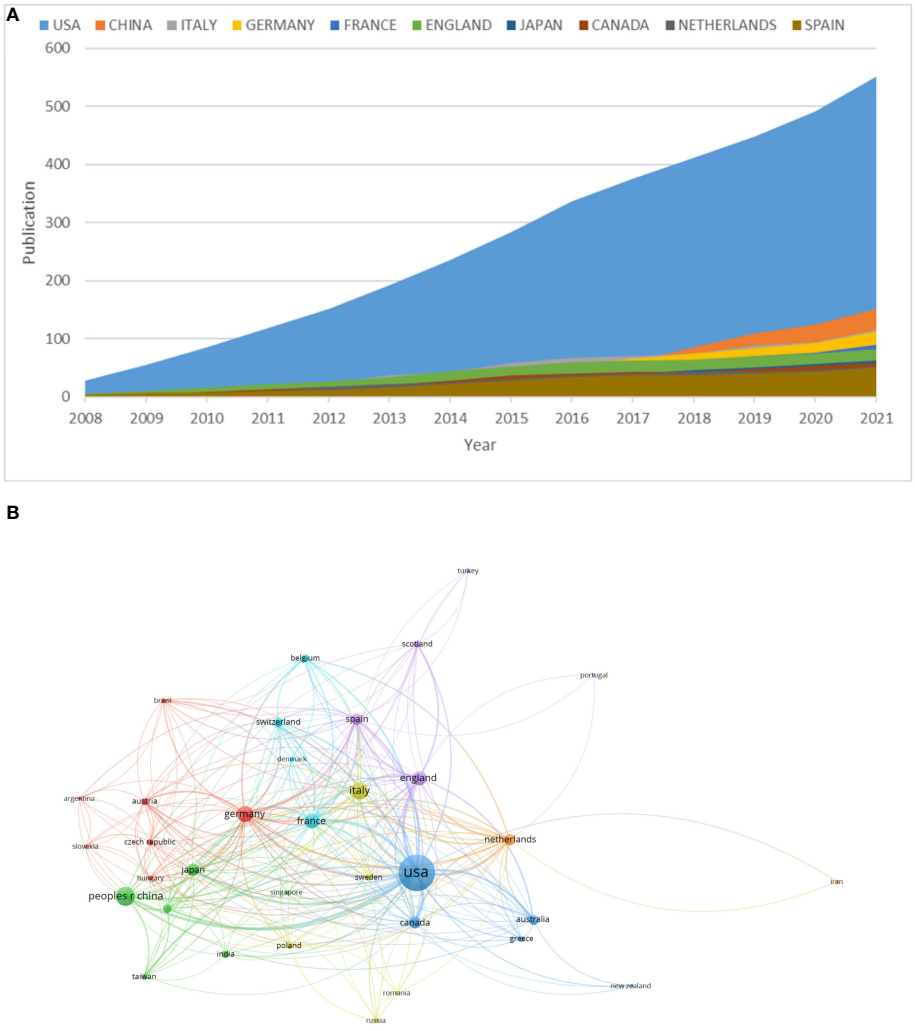

FIGURE 3
(A) The changing trend of the annual publication counts in the top 10 countries. **(B)** The country citation network visualization map generated by VOS viewer (version5.8 R3). USA, The United States of America.

TABLE 2 The top 10 productive institutions ranked by the numbers of publications.

Rank	Institutions	Countries	Article count	H-index	Total citations	Average citation per article
1	University of Texas system	United States	98	36	3,551	36.23
2	UTMD Anderson Cancer Center	United States	84	33	2,975	35.42
3	Harvard University	United States	70	26	3,515	50.21
4	University Of California System	United States	54	26	2,009	37.2
5	Unicancer	France	48	27	4,603	95.9
6	Memorial Sloan Kettering Cancer Center	United States	47	23	1,857	39.51
7	National Institutes of Health (NIH)	United States	42	23	2,006	47.76
8	Dana Farber Cancer Institute	United States	36	18	1,715	47.64
9	National Cancer Institute (NCI)	United States	35	21	1,808	51.66
10	Inserm	France	32	15	3,119	97.47

institution with largest TLS was Memorial Sloan Kettering Cancer Center (TLS=204), and all other institutions had less than 100 TLS.

B. The institutions' citation network visualization map generated by VOS viewer (version5.8 R3).

Authors and co-cited authors

A total of 8,422 authors appeared in the 1,229 articles. The top 10 most productive authors in the publications were listed in Table 3. Lacouture M.E from United States contributed the most articles (18 articles) and the highest H-index (13). Robert C from Canada had the most average citation per articles (188.55 times per articles).

Figure 5A illustrated the collaboration between the authors. Prolific authors like Lacouture M.E and Robert C had the active network of collaborators. The authors' co-citation network included 188 items, 5 clusters and 7,936 links (Figure 5B). The top three authors with largest TLS were Robert C(TLS=5,187), Long G.V(TLS=3,586) and Hodi F.S(TLS=3,286).

Journals and co-cited journals

A total of 204 journals published articles on precision cancer medicine-related rashes. The top 10 journals were listed based on a comprehensive quality assessment (Table 4). As shown in the table, 299 articles were published in the top 10 journals, accounting for 24.3% of the included articles. Cancers (IF 2021 = 6.639) came out the most articles (count:70 pieces), the following were Frontiers in Oncology (IF 2021 = 6.244) and Oncotarget (this journal was not in the latest JCR). Of the top 10 journals, five were from the United States, three from England and two from Switzerland. Clinical Cancer Research and Cancer Research had the highest H-index (22). Meanwhile Clinical Cancer Research had the most total citations (1,709 times) and highest IF (IF 2021 = 12.531).

Figure 6 was a dual-map overlay of the relevant journals, which revealed the citation relationships of the journal among related fields through visualization. The labels on the map represent the field to which the journal belongs. The left side of the map represented the field of citing literature, and the right side represented the field of cited literature. Different colors represented different citation paths. The figure identified three main citation paths, including two green paths and one orange path. The orange line showed that the includes articles were mostly distributed in the fields of Molecular, Biology and Immunology, while the cited articles were mostly distributed in the fields of Molecular, Biology and Genetics. The green path indicated that the articles included in the analysis were mostly distributed in the fields of Medicine, Medical and Clinical, while the cited articles were mostly distributed in the fields of Molecular, Biology, Genetics, Health, Nursing and Medicine. The determination of citation path can represent the causal relationship of citation. The citing literature can be regarded as the applied research in this field, while the cited literature can be regarded as the research basis in this field.

Citations and co-cited citations

The top 10 related articles with the most citations are shown in Table 5. Journal of Clinical Oncology and Lancet Oncology had a huge scientific impact on researchers and scholars in this field, with more than half of the top 10 most-cited articles published in these journals. All the top 10 references were co-cited more than 320 times. The study by Michot.JM et al. (2016), published in European Journal of Cancer, was the most cited article with 1,122 times by far. CiteSpace V (version5.8 R3) was used to search for citation burstiness, and a total of 25 references with the highest citation bursts were found in Figure 7. References with citation bursts first appeared in 2008, while the burst originated from a paper in 2004. About 60% of the references were cited between 2012 and 2016. The most recent reference with citation burst was observed in 2019, and this burst is still ongoing.

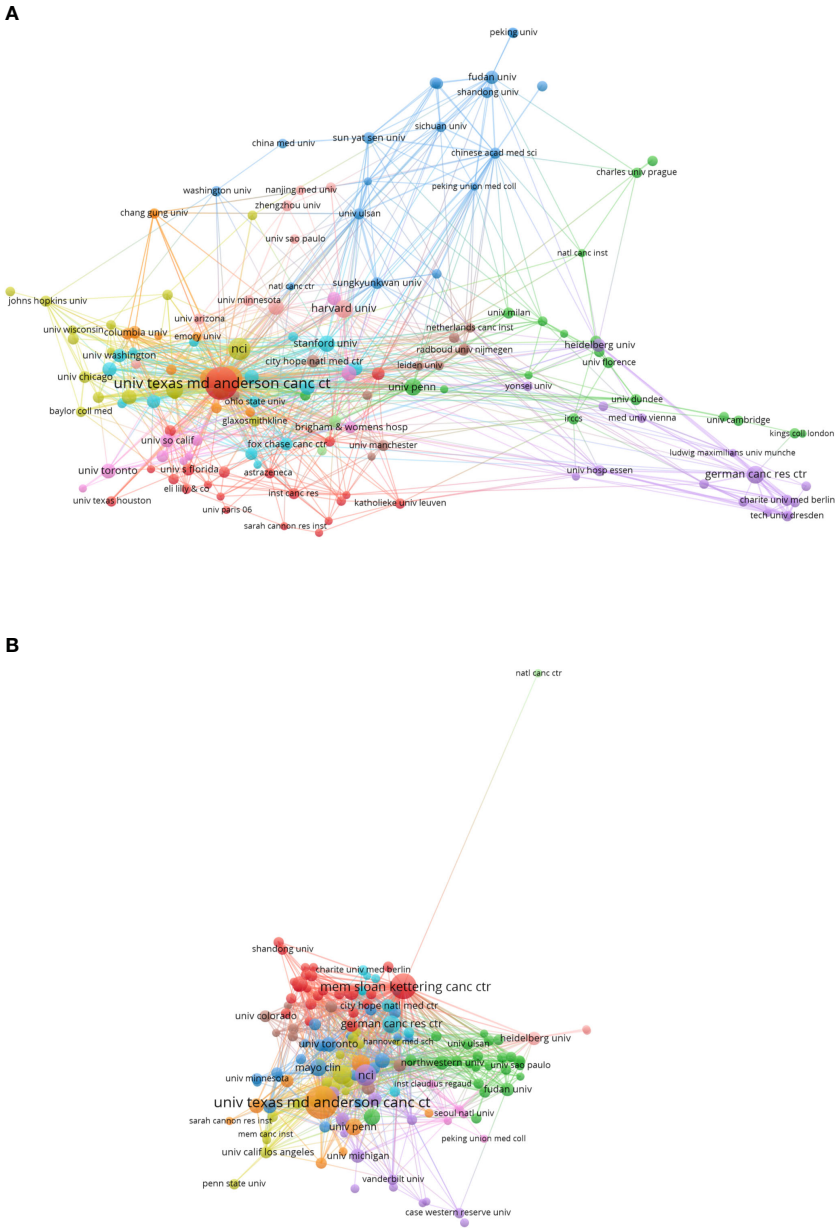

FIGURE 4
(A) The institutions' collaboration network visualization map generated by VOS viewer (version5.8 R3) **(B)** The institutions' citation network visualization map generated by VOS viewer (version5.8 R3).

Keywords analysis of research hotspots

We extracted keywords from the titles and abstracts of 1,229 included articles. Keywords that appeared more than 50 times were used to generate a visual map through VOS viewer (version5.8 R3), which contained 32 keywords (Figure 8A). Cluster analysis was conducted for high-frequency keywords (occurrences greater than 50 times). There were 32 nodes and 461 links in the visualization map, and the high-frequency keywords were grouped into three clusters (Cluster 1: Red; Cluster 2: Green; Cluster 3: Blue).

Cluster 1 was the largest, and the most frequently appeared keywords were melanoma (215 times), cancer (185 times) and expression (144 times). The main keywords of cluster 2 were targeted therapy (194 times), chemotherapy (127 times) and open label (109 times). For cluster 3, prominent keywords were phase-II (86 times), efficacy (71 times) and management (71 times). Then, we assigned keywords to the timeline, and

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TABLE 3 The top 10 most productive authors in publications.

Rank	Author	Article count	H-index	Countries	Total citations	Average citation per article	Institutions
1	Lacouture ME	18	13	United States	491	27.28	Memorial Sloan-Kettering Cancer Center
2	Robert C	11	9	Canada	2,074	188.55	Minist Environm & Lutte Changements Climat
3	DiGiovanni J	11	8	United States	347	31.55	University of Texas Austin
4	Wang Y	9	5	China	165	18.33	Chinese people's liberation army general hospital
5	Li J	8	5	China	523	63.38	Fudan University
6	Zhang Q	8	5	China	141	17.63	Nanjing Medical University
7	Ascierto PA	7	4	Italy	840	120	IRCCS Fondazione Pascale
8	Belum VR	7	7	India	122	17.43	ICRISAT
9	Dummer R	7	6	Switzerland	636	90.86	University of Zurich
10	Loquai C	7	3	Germany	62	8.86	Johannes Gutenberg University of Mainz

obtained two time-varying visual maps of keywords through VOS viewer (version5.8 R3) and CiteSpace V (version5.8 R3). In Figure 8B, the occurrence of keywords has changed from blue to red color over time, indicating that research hotspots related to Melanoma and Immunotherapy have emerged in recent years. Figure 9A shows the clustering of keywords into six clusters by CiteSpace V (version5.8 R3), which reflects the hot research directions in recent years: Keratosis prevention migration, Hand-Foot Syndrome, Stage III Melanoma, Cutaneous adverse event, Next-generation Sequencing, and Cell death.

Burst keywords are another important data to reflect research hotspots and academic frontiers (Figure 9B). The red part indicated that the keywords show a trend of blowout at this stage. We found that there were still keywords emerging until the end of 2021, such as ipilimumab, immunotherapy, tumor microenvironment, association, checkpoint inhibitor, indicating that these research directions had been highly concerned in recent years and might become hot spots and directions of future research.

Discussion

In this study, we examined the global scientific output associated with researches on precision cancer medicine-related rashes from 2008 to 2021 using a bibliometric analysis. As shown in Figure 2, the number of global publications on precision cancer medicine-related rashes increased significantly from 2008 to 2021. Before 2013, the global related publications showed a gradual growth trend. The number of publications tends to stabilize between 2013 and 2019. And in 2021, there was a substantial increase. Therefore, it is speculated that this field may enter a golden age in the next few years.

In terms of country analysis, the United States was the country with the largest output in this field, and the number of its publications is much higher than that of other countries. At the same time, the United States had the most citations, the highest H-index, and the largest TLS, suggesting that the quality and influence of articles published by the United States is higher, indicating that the United States is in a dominant position in this field, followed by Germany and France. And notably, although China ranked second in the number of publications, the citations, H-index and TLS were the bottom, pointing that Chinese scholars should pay more attention to the quality of academic articles.

Among the top 10 institutions, except for 2 laboratories from France, the remaining 8 institutions were all from the United States. This may be the reason why the United States has published the most correlational researches on precision cancer medicine-related rashes. And these results also showed that the establishment of first-scale colleges and research institutions provided a crucial foundation to promote the national academic status.

On the list of the top 10 most prolific scholars, two were from the United States, three were from China, and other five were from Canada, Italy, India, Switzerland and Germany, respectively. Lacouture ME from Memorial Sloan-Kettering Cancer Center, Robert C from Minist Environm & Lutte Changements Climat and DiGiovanni J from University of Texas Austin contributed the most publications. Figure 5A is an assessment of the relationship between projects by the number of co-authored documents, revealing the collaborative relationship between authors. There is a close cooperation between authors labeled with the same color. Figure 5B illustrates the relationship between authors based on the number of citations they have received together. Authors

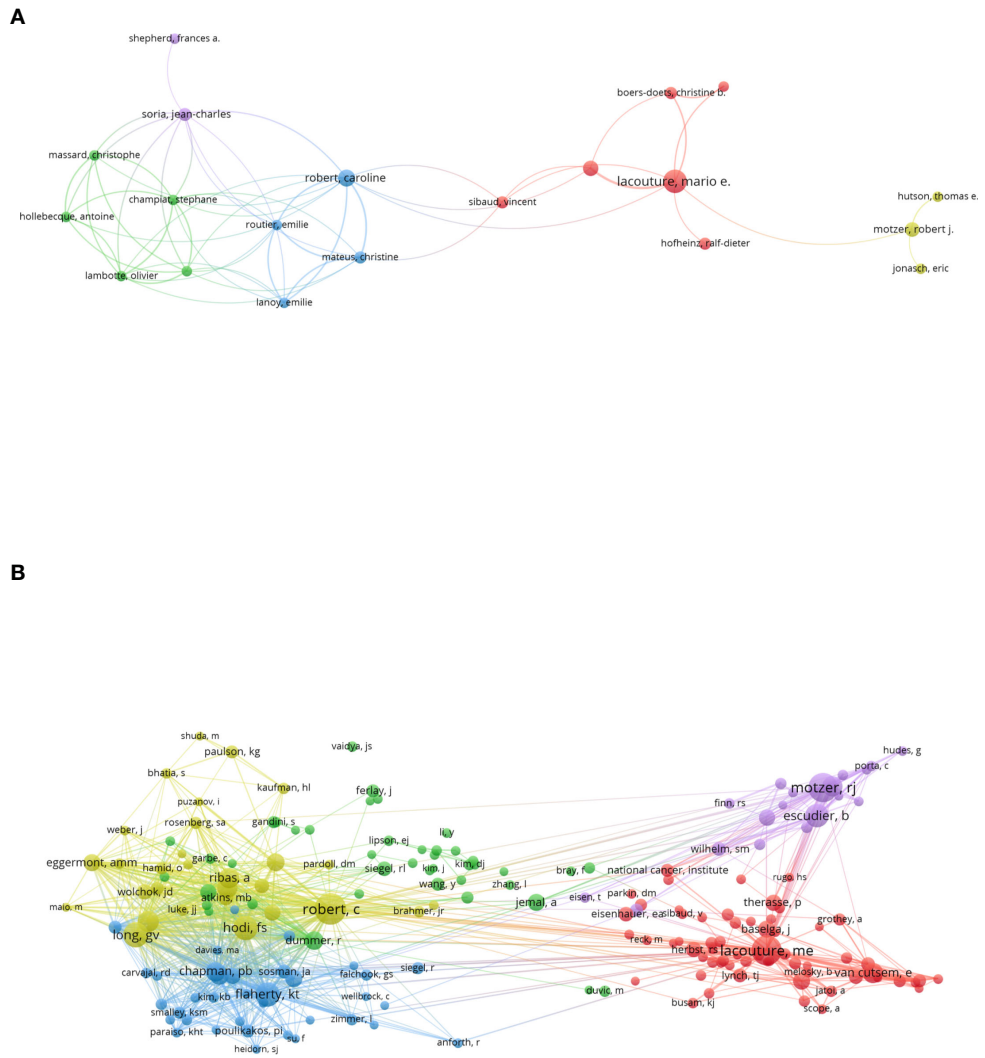

FIGURE 5
(A) The authors' collaboration network visualization map generated by VOS viewer (version5.8 R3). **(B)** The authors' co-citation network visualization map generated by VOS viewer (version5.8 R3).

TABLE 4 The top 10 journals of research ranked by publication number.

Rank	Journal Title	Country	Count	IF (2021)	Quartile in category (2021)	H-index	Total citations
1	Cancers	Switzerland	70	6.639	Q2	11	395
2	*Frontiers in Oncology*	Switzerland	32	6.244	Q3	9	700
3	*Oncotarget*	United States	32	/	/	14	460
4	*Clinical Cancer Research*	United States	27	12.531	Q1	22	1,709
5	*Melanoma Research*	United States	27	3.599	Q2/3	10	374
6	*Cancer Research*	United States	26	12.701	Q1	22	1,668
7	*Investigational New Drugs*	United States	25	3.85	Q2/3	13	712
8	*Oncogene*	England	21	9.867	Q1	13	701
9	*British Journal of Cancer*	England	20	7.64	Q1	14	701
10	*European Journal of Cancer*	England	19	9.162	Q1	12	1,502

IF, Impact factor.

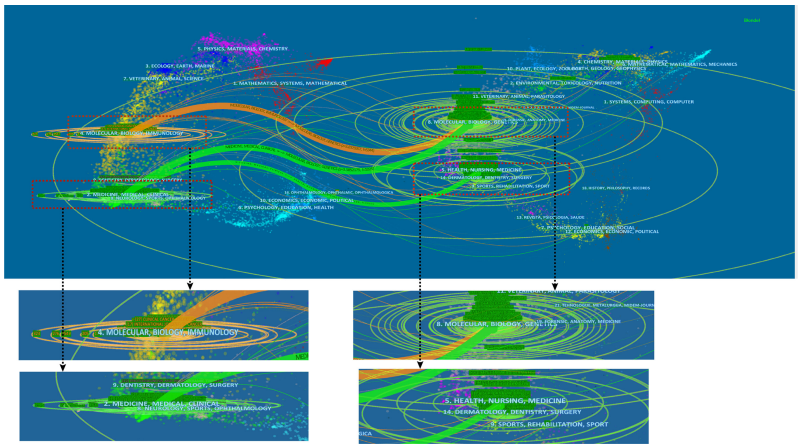

FIGURE 6
A dual-map overlay of the relevant journals generated by CiteSpace V (version5.8 R3).

marked with the same color have similar research fields, and larger nodes predict dominance in this area for that author. This analysis may help new researchers to better understand existing partnerships and identify important/potential collaborators in the field. Lacouture ME and Robert C had active collaborator networks, and Robert C had the highest co-citation link strength. They had played an important leadership role in this field, more important publications related to precision cancer medicine-related rashes are more likely to be published by these authors and their teams.

In terms of top journals, those listed in Table 4, such as Clinical Cancer Research, Cancer Research, Oncogene, European Journal of Cancer, British Journal of Cancer may be core journals for research publications in precision cancer medicine-related rashes. This result guides scholars to submit more manuscripts to these journals. Among the top ten journals, there are two journals with IF greater than 10.0, namely Cancer Research (IF2021, 12.701) and Clinical Cancer Research (IF2021, 12.531). Five of the top ten journals, including Cancers (IF2021, 6.639), Frontiers in Oncology (IF2021, 6.244), Oncogene (IF2021, 9.867), British Journal of Cancer (IF2021, 7.640) and European Journal of Cancer (IF2021, 9.162), had an IF between 5.0 and 10.0. Overall, publishing articles related to precision cancer medicine-related rashes in high-IF journals remains a challenge.

"References with citation bursts" means that the corresponding research is frequently cited within a certain period. This indicator suggests that these publications have attracted considerable attention in the scientific community, and reflects the hot spots and dynamic changes in the field of precision cancer medicine-related rashes. The first citation burst appeared in 2008 and stemmed from the article published by Cunningham D et al. in 2004 (11), which showed that the clinical response rate of colon cancer patients with skin rash after cetuximab treatment was significantly higher than that of patients without skin reaction. This citation burst gradually drew scholars' attention to the study on the relationship between precision cancer medicine-related rashes and clinical efficacy.

Between 2012 and 2016, about 60% of the references experienced citation bursts. Notably, the bursts of three studies are still ongoing. Among them, Larkin J et al. (12) (2015) found in the phase 3 clinical trial NCT01844505 that the incidence of rashes was 40.3% (4.8% for grade 3 or 4) in melanoma patients treated with the combination of nivolumab and ipilimumab, and the incidence of rashes in patients treated with nivolumab or ipilimumab alone was 25.9% (0.6% for grade 3 or 4) and 32.8% (1.9% for grade 3 or 4). Robert C et al. (2015) found in clinical trial NCT01721772 (13)that rashes occurred in 15.0% of melanoma patients treated with nivolumab (0.5% for grades 3 or 4, resolved rapidly with study treatment delay and/or administration of glucocorticoids), while the incidence of rashes was 2.9% in dacarbazine-treated patients with no grade 3 to 4 rashes occurred; in clinical trial NCT01866319 (14), patients with advanced melanoma treated with pembrolizumab (10 mg/kg q2w or q3w) had a longer time until the onset of the first grade 3 to 5 adverse events. The incidence of permanent discontinuation due to the treatment-related adverse events was lower in the pembrolizumab group than in the ipilimumab group. And the occurrence of rashes in each pembrolizumab group and ipilimumab group was 14.7% (q2w), 13.4% (q3w), and 14.5%, respectively. No severe grade 3-5 rashes occurred in the pembrolizumab group, compared with 0.8% in the ipilimumab group. It is noteworthy that the most recent bursts began in 2019 and remain ongoing. This is mainly related to two articles published by Bray F et al. (15) (2018) in CA Cancer J Clin and Migden MR et al. (16) (2018) in N Engl J Med.

Through the analysis of frequently occurring keywords, we can further understand the changing trends and main topics

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TABLE 5 The top 10 related articles with the most citations.

Title	First author	Journal	Year	Citations	Main conclusion
Immune-related adverse events with immune checkpoint blockade: a comprehensive review	Michot.JM	European Journal of Cancer	2016	1122	They summarized the various manifestations and management of immune-related adverse events (irAEs) caused by anti-CTLA-4 antibodies and anti-PD-1/PD-L1 antibodies. Vitiligo is the most common dermatologic irAEs in melanoma patients, others including rash, erythema, Stevens-Johnson syndrome and toxic epidermal necrosis (SJS/TEN). For the treatment of grade I-II, topical corticosteroids combined with oral antipruritic drugs are effective (skin infection must be excluded before steroid applying); for grade III-IV, a skin biopsy is necessary and systemic steroids are appropriate. And pointed out that after a typical 2-4 week course of full steroid doses, steroids must be tapered over a period of at least 1 month to avoid irAEs recurrence.
Lapatinib Combined With Letrozole Versus Letrozole and Placebo As First-Line Therapy for Postmenopausal Hormone Receptor-Positive Metastatic Breast Cancer	Johnston.S	Journal of Clinical Oncology	2009	755	They found that in HR-positive, HER-2-positive metastatic breast cancer patients, letrozole combined with lapatinib can significantly improve clinical benefit rates, prolong median PFS, and reduce the risk of disease progression compared with letrozole plus placebo. But it also has a higher incidence of rashes (grade1-4, 44% vs 13%; grade3-4, 1% vs 0%).
Safety profiles of anti-CTLA-4 and anti-PD-1 antibodies alone and in combination	Boutros.C	Nature Review Clinical Oncology	2016	533	They described the adverse event profiles and mechanism of CTLA-4 and PD-1-targeted checkpoint inhibitors in melanoma patients, including rash, pruritus, enterocolitis, diarrhea, anorexia, fatigue, hypophysitis, and more. Concomitant use of both drugs was associated with a higher rate of irAEs and a wider range of adverse events. The possible association of irAEs with the clinical benefit of their treatment has not been fully elucidated. In management, grade 1-2 irAEs that do not interfere with daily live activities usually do not require dose omission or discontinuation. For patients with persistent grade 2 or more severe irAEs, symptoms should be treated with corticosteroids, and dose skipping or discontinuation is also required.
Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): a proof-of-concept, multicentre, open-label, phase 2 trial	Sartore-Bianchi.A	Lancet Oncology	2016	481	They used trastuzumab and lapatinib dual-targeted therapy to treat 27 patients with refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic colorectal cancer. The results showed that the objective response rate was 30% (CR 4%, PR 26%), 44% of patients had stable disease, 48% (13/27) of patients developed rash (1 patient had a grade 3 rash), and there were no treatment-related grade 4 and 5 adverse events.
MEK162 for patients with advanced melanoma harbouring NRAS or Val600 BRAF mutations: a non-randomised, open-label phase 2 study	Ascierto.PA	Lancet Oncology	2013	468	They evaluated the efficacy of MEK162 in patients with advanced melanoma with NRAS or Val600 BRAF mutations. Median follow-up was 3.3 months (range 0.6-8.7; IQR 2.2-5.0). No patient had a CR. Six (20%) of 30 patients with NRAS-mutant melanoma had a PR (3 confirmed) and 8 (20%) of 41 patients with BRAF-mutated melanoma had a PR (2 confirmed). Rash occurred in 6 (20%) patients with NRAS-mutant melanoma and 16 (39%) patients with BRAF-mutant melanoma. There were no deaths from treatment-related causes.
Anti-PD-1 and Anti-CTLA-4 Therapies in Cancer: Mechanisms of Action, efficacy, and Limitations	Seidel.JA	Frontiers In Oncology	2018	455	They summarized the advantages and limitations of immune checkpoint inhibitors in tumor therapy, as well as immunotherapy-related adverse event and management. Patients treated with anti-CTLA-4 had a higher incidence of side effects than those treated with anti-PD-1. Certain treatment-related autoimmune reactions such as rash and vitiligo were associated with better outcomes.
Regorafenib plus best supportive care versus placebo plus best supportive care in Asian patients with previously treated metastatic colorectal cancer (CONCUR): a randomised, double-blind, placebo-controlled, phase 3 trial	Li.J	Lancet Oncology	2015	431	They demonstrated an OS benefit from regorafenib in Asian patients with refractory metastatic colorectal cancer in a Phase 3 clinical trial. The most common grade 3 or higher adverse events associated with regorafenib were HFSR(16% in the regorafenib group), and they found the highest incidence of HFSR, rash, and fatigue within one to two weeks of initial treatment.

(Continued)

TABLE 5 Continued

Title	First author	Journal	Year	Citations	Main conclusion
BRAF/NRAS Mutation Frequencies Among Primary Tumors and Metastases in Patients With Melanoma	Colombino.M	Journal Of Clinical Oncology	2012	343	They explored the relative frequency of genetic factors(BRAF/NRAS/p16CDKN2A) known to play an important role in melanoma development, and their distribution among different melanoma tissues and disease progression sites by sequencing DNA from tissue samples.
SEARCH: A Phase III, Randomized, Double-Blind, Placebo-Controlled Trial of Sorafenib Plus Erlotinib in Patients With Advanced Hepatocellular Carcinoma	Zhu AX	Journal Of Clinical Oncology	2015	335	They evaluated the efficacy of sorafenib plus erlotinib for advanced liver cancer and concluded that the combination did not improve survival in patients with advanced liver cancer. The incidence of serious adverse reactions was 58% (sorafenib/erlotinib) and 54.6%(sorafenib/placebo). The incidences of rash/descaling(51.9%vs40%),anorexia(42.5% vs37.2%)and diarrhea(76.2%vs59.4%)were higher in the sorafenib/erlotinib group, and alopecia (23.7%vs12.7%) and HFSR (47.6%vs38.1%) were higher in the sorafenib/placebo group.
Hippo-Independent Activation of YAP by the GNAQ Uveal Melanoma Oncogene through a Trio-Regulated Rho GTPase Signaling Circuitry	Feng.XD	Cancer Cell	2014	320	They found that transcriptional coactivator YAP was a suitable therapeutic target for uveal melanoma. They demonstrated that YAP activation represented a key factor in GNAQ-induced tumorigenesis and that inhibition of YAP function might represent a pharmacological intervention strategy.

development in this field. As shown in the keyword clustering diagram in the Figure 8A, all the keywords of research on precision cancer medicine-related rashes could be divided into 3 categories. Cluster 1 mainly focused on the therapeutic targets of tumors, and the prominent keywords were melanoma, cancer, and expression. Cluster 2 was primarily about clinical application of targeted therapy, and the main keywords were targeted therapy, chemotherapy and open label. Cluster 3 mainly focused on the efficacy of precision cancer medicine including targeted and immunotherapy, and the management of related adverse events. The primary keywords were phase-II, efficacy and management. As a rapidly developing field, precision cancer medicine started from the basic research of tumorigenesis mechanism, and the development and clinical application of the drug were correspondingly carried out, and the related adverse events also followed. Therefore, when researching the mechanism of precision cancer medicine-related rashes in the future, we should also focus on exploring how to effectively manage it without reducing the anti-tumor intensity or terminating anti-tumor therapy.

According to the timeline view and the top 25 most cited keywords in publications exported by CiteSpace V (version5.8

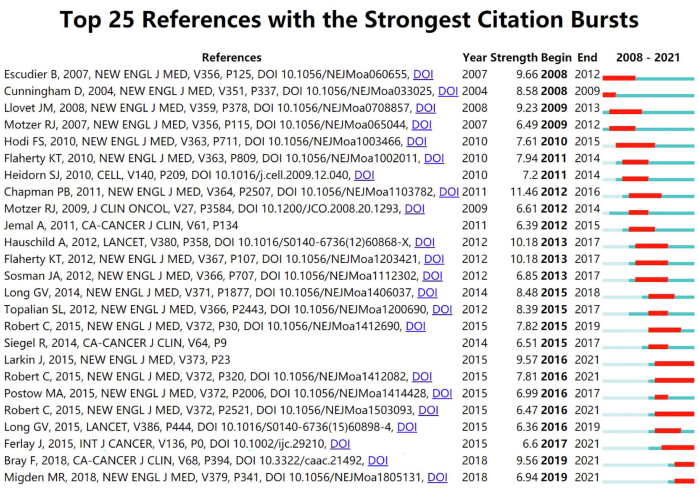


FIGURE 7
The top 25 references with the strongest citation. (The green line segment represents the time interval, and the red line segment represents the active time).

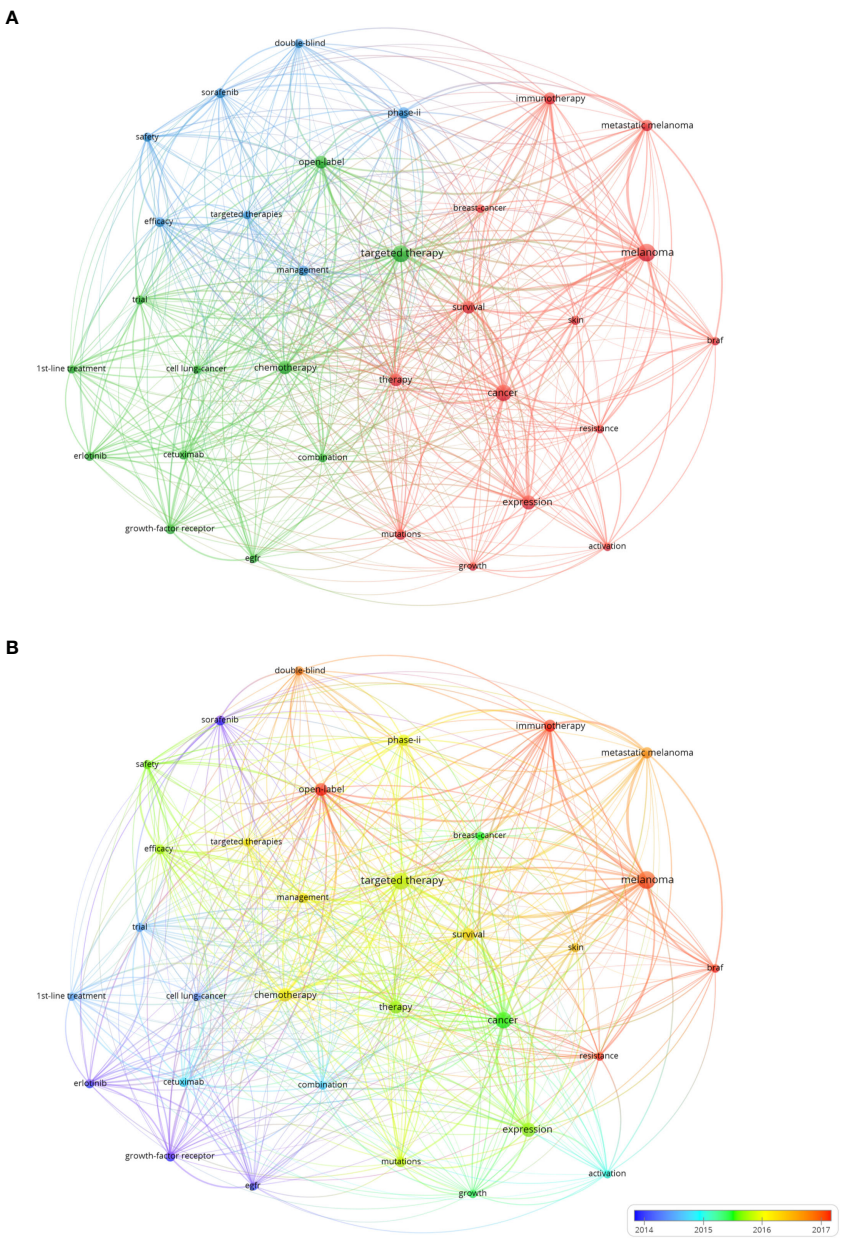
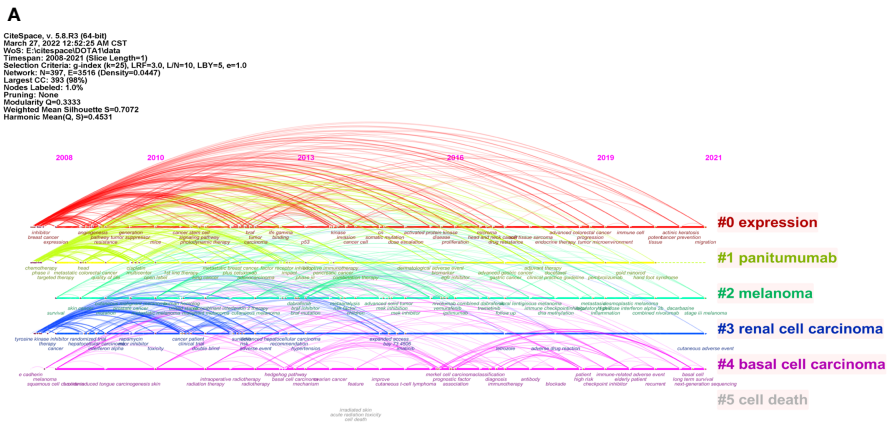


FIGURE 8
(A) The network visualization map of keywords generated by VOS viewer (version5.8 R3). **(B)** The visualization map of keywords over time by VOS viewer (version5.8 R3).

R3), it can be seen that in the past few years, there has been a certain research foundation for targeted therapy-related cutaneous adverse events (AEs): Hand-foot-skin reaction (HFSR), in particular, is one of the most common targeted therapy-induced AEs, will lead to treatment interruption or failure. Some achievements have been made in the diagnosis, prevention, evaluation, management and possible mechanism of HFSR (17–22). At the same time, four potential research hotspots and frontiers can also be predicted, as follows: "checkpoint inhibitors", "cutaneous adverse events", "association" and "tumor microenvironment".

(1) Checkpoint inhibitors: Since the discovery of immune antitumor responses was first published about a century ago (23), the research on tumor immunotherapy has grown step by step. Immunotherapy has gradually become a viable treatment option for a variety of tumors over the past 30 years. Anti-cytotoxic T lymphocyte-associated protein 4 (CTLA-4) was approved in 2011 for the treatment of advanced melanoma,



B

Top 25 Keywords with the Strongest Citation Bursts

Keywords	Year	Strength	Begin	End	2008 - 2021
renal cell carcinoma	2008	7.4	2008	2013	
growth factor receptor	2008	5.3	2008	2010	
monoclonal antibody	2008	5.12	2008	2011	
transgenic mice	2008	5.07	2008	2010	
cell lung cancer	2008	4.99	2008	2010	
antitumor activity	2008	4.58	2008	2014	
stem cell	2008	4.31	2008	2012	
gene expression	2008	4.08	2008	2012	
phase ii trial	2008	3.85	2008	2010	
interferon alpha	2008	6.41	2009	2013	
in vivo	2008	6.27	2009	2012	
phase i	2008	4.95	2010	2013	
sorafenib	2008	5.07	2011	2013	
sunitinib	2008	4.45	2012	2016	
phase iii	2008	3.93	2013	2014	
open label	2008	6.71	2016	2018	
ipilimumab	2008	5.06	2016	2021	
vemurafenib	2008	4.11	2016	2019	
prognostic factor	2008	3.96	2016	2018	
immunotherapy	2008	4.76	2017	2021	
dabrafenib	2008	4.55	2017	2019	
signaling pathway	2008	3.91	2017	2018	
tumor microenvironment	2008	4.97	2019	2021	
association	2008	4.27	2019	2021	
checkpoint inhibitor	2008	4.14	2019	2021	

FIGURE 9
(A) Timeline view of keywords from publications generated by CiteSpace V (version5.8 R3). **(B)** The top 25 keywords with the strongest citation bursts of publications (The green line segment represents the time interval, and the red line segment represents the active time).

and other immune checkpoint inhibitors (ICIs) were quickly approved by the Food and Drug Administration (24). Today, the profound and long-lasting antitumor effects of immune checkpoint inhibitors have revolutionized oncology and altered the prognosis of many cancers (25–28).

Notwithstanding, resistance to ICIs limits the number of patients who can achieve a lasting response. With the study of the mechanisms of tumor immune evasion after ICIs treatment, we have improved our understanding of the basis for efficacy and resistance, and we are achieving even more impressive success through different combinatorial strategies, including combinations of different immune checkpoint inhibitors, or combination with targeted therapies, chemotherapy and so on. For example, the 5-year OS of the combination of PD-1 inhibitor

nivolumab and CTLA-4 inhibitor ipilimumab was unprecedented higher than 50% (29). And OS, progression-free survival (PFS), and objective response rate (ORR) were significantly improved in patients receiving the combination of ICIs and anti-VEGF, although with the expense of increased AEs (27). Published trials of combinations of BRAF/MEK inhibitors and ICIs have produced conflicting results in PFS, and the optimal sequence of BRAF/MEK inhibitors and ICIs is still pending on reliable clinical data (30, 31). In addition, novel combinations of immune checkpoint inhibitors (ICIs), such as cytokines, oncolytic viruses, TLR9 agonists, HDAC inhibitors, DNMT inhibitors, etc, are under investigation (32–35).

On the road to treat, these new methods and the individualized application of combination therapy have important research significance to the best benefit-risk ratio in clinical practice, which can improve the long-term survival rate of cancer patients further.

(2) Cutaneous adverse events: Along with the use of various targeted therapies and immunotherapies, new adverse events have emerged, including cutaneous toxicities (up to 30-60%), which range from limited morbilliform eruptions, maculopapular rash to diffuse bullous rash and even dermatological emergencies with high mortality rates such as Stevens-Johnson Syndrome (SJS), Toxic Epidermal Necrolysis, and drug reaction with eosinophilia and systemic symptoms (DRESS) (36). These cutaneous adverse events will not only greatly impact the patient's quality of life, they can also lead to cessation or discontinuation of treatment, and affect patient survival (37).

The study found that compared with chemotherapy and targeted therapy, patients treated with ICIs had an increased risk of any related adverse events (RR, 2.65; 95% CI, 1.84-3.83; $P < 0.00001$), including rash (1.58; 0.98-2.54) and other cutaneous adverse events (38). Compared with PD-1 ICIs (34-42%) or PD-L1 ICIs monoclonal treatment (20%), CTLA-4 ICIs monoclonal treatment was associated with a higher incidence of cutaneous adverse events (44-59%), and the incidence of combination therapy was the highest (59-72%) (39). And treatment adjustments due to cutaneous adverse events were more common in patients treated with targeted therapies and ICIs than with chemotherapy. The cutaneous adverse events that led to most treatment modifications was skin rash (40). To date, several guidelines for the management of immunotherapy-associated rashes have developed, mainly based on case reports, series, experience, and expert consensus. However, the effectiveness of these treatments is rarely reported (41, 42).

The occurrence and treatment of precision cancer medicine-related rashes complicate antitumor therapy, and therapeutic measures must be taken as soon as possible to reduce their severity and duration. Therefore, accurate diagnosis and effective management are important bottlenecks to be solved.

(3) Association: In recent years, more and more studies have shown that the occurrence of irAEs means that patients benefit from immunotherapy, including non-small cell lung cancer (43, 44), renal cell carcinoma (45), cutaneous melanoma (46), etc. Johnston.S et al. (47)found that in HR-positive, HER-2-positive metastatic breast cancer patients, letrozole in combination with lapatinib significantly improved clinical benefit rates, prolonged median PFS, and reduced risk of disease progression compared with letrozole in combination with placebo, while also resulting in a higher incidence of rash (grades 1-4, 44% vs. 13%; grades 3-4, 1% vs. 0%). In patients with different tumors such as melanoma and NSCLC using PD-1 immune checkpoint inhibitors, teams such as Freeman-Keller M (46), Akano Y (48), Quach HT (49), Lee YJ (50), Aso M (51), Bottlaender L (52) have observed that ORR, PFS and OS of patients who developed skin reactions such as rash were significantly better. A systematic review of 137 immunotherapy studies showed that the occurrence of vitiligo did not have an advantage in long-term PFS or OS, while the occurrence of remaining irAEs proved to be a survival advantage in long-term follow-up (53, 54), mainly in terms of prolonged PFS and OS and significant improvement in ORR. There are no articles reporting that patients who developed rash after receiving precision cancer medicine had worse disease remission rates currently. The mechanism of precision cancer medicine-related rashes has not been fully elucidated, but its clinical significance has attracted extensive attention. At present, most retrospective studies have confirmed this conclusion, which needs to be further verified in larger prospective clinical studies.

(4) Tumor microenvironment: ICIs are monoclonal antibodies that block receptors, which lead to the activation of immune cells in the tumor microenvironment (55, 56), and the benefits of cancer therapy are accompanied by autoimmune side effects known as irAEs. IrAEs caused by ICIs are thought to occur through several immunologic pathways. In the physiologic state, CTLA-4 is involved in thymic maturation of T cells and downregulating T cell activation, while the PD-1 pathway is involved in the induction and maintenance of peripheral tolerance against self-reactive T cells. When these pathways are pharmacologically blocked, T cell responses are promoted, leading to both antitumor responses and the proliferation of self-reactive T cells with resultant autoimmunity (39). These reactions typically affect the skin, colon, liver, lungs, endocrine organs, and joints (56, 57). Another idea is that cross-reactive T cells (T cells that bind to tumor and irAEs target tissues) may play a role, mechanism involves cross reactivity between antigens on the target tumor cells and self-antigens on normal host tissues. Vitiligo in particular has been linked to cross-reactivity between melanoma-associated antigens and melanocytes, both of which may become targets of the ICI associated immune response (58, 59). Bullous dermatitis is a type of immune-associated rash, and a study (60) found that basement membrane protein BP180, which targets newborn bullous dermatitis, may mediate this reaction. In addition, cytokines and chemokines may also be important mediators in the pathogenesis of irAEs (61), but there is a lack of high-level evidence to support this.

Strengths and limitations

Our study is the first time to use bibliometric analysis and visualization tools to analyze global trends of precision cancer medicine-related rashes, which systematically demonstrates the evolution, status, and frontiers of related researches. The limitations of this study are as follows: first, we only retrieved and collected literature data from the WOSCC database, which may miss important studies in PubMed, Embase and other databases. Second, the study only included English literature, and important researches published in other languages may be ignored. Finally, only the journal's impact factor and quartile in category were evaluated, and the quality of the articles included in the study was not assessed.

Conclusion

To conclude, the study of precision cancer medicine-related rashes is in a developmental stage, and the number of relevant publications will increase rapidly. The United States has the largest quantity of studies on this topic and the highest quality and impact of its articles, is playing a guiding role in this field. At present, the research focus is gradually shifting to tumor immunotherapy, "checkpoint inhibitors", "skin adverse events", "association" and "tumor microenvironment" may become the future research hotspots.

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

FZ contributed to conceptualization, visualization and writing-original draft, review and editing. RY contributed to conceptualization, visualization and writing-original draft, review and editing.SC contributed to conceptualization, funding acquisition and writing-original draft, review and editing. SZ contributed to writing-review and editing. LS contributed to writing- review and editing. ZX contributed to writing-review and editing. YZ contributed to writing-review and editing. SD contributed to writing-review and editing. GZ contributed to writing-review and editing. QS contributed to supervision, funding acquisition and writing-review and editing. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the following research grants from clinical (gastric cancer) cooperation pilot project of Chinese and Western Medicine for Major; and Difficult Diseases and Zhejiang Traditional Chinese Medicine of Science and Technology Program (No. 2020ZA053).

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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frontiers | Frontiers in Immunology

TYPE Case Report
PUBLISHED 26 August 2022
DOI 10.3389/fimmu.2022.989966

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Immunology

RECEIVED 11 July 2022
ACCEPTED 08 August 2022
PUBLISHED 26 August 2022

CITATION
Li G, Gong S, Wang N and Yao X (2022) Toxic epidermal necrolysis induced by sintilimab in a patient with advanced non-small cell lung cancer and comorbid pulmonary tuberculosis: A case report.
Front. Immunol. 13:989966.
doi: 10.3389/fimmu.2022.989966

Toxic epidermal necrolysis induced by sintilimab in a patient with advanced non-small cell lung cancer and comorbid pulmonary tuberculosis: A case report

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Immune checkpoint inhibitors (ICIs) have had a revolutionary effect on the treatment of patients with advanced non-small cell lung cancer (NSCLC), especially squamous cell lung cancer. However, ICIs may cause associated immune-related adverse events (ir-AEs). No case of sintilimab-induced toxic epidermal necrolysis (TEN) has been reported. In this report, we discussed a patient with advanced NSCLC and comorbid pulmonary tuberculosis who underwent immunotherapy and chemotherapy as neoadjuvant therapy and anti-tuberculosis therapy concurrently. Partial response (PR) of the tumor was achieved after three cycles of neoadjuvant therapy without cutaneous toxicities. Video-assisted thoracoscopic surgery (VATS) left lower lobectomy was performed successfully. Sintilimab and chemotherapy were administered as adjuvant therapy, after which the patient suffered severe TEN that rapidly progressed to cover >50% of the skin. TEN was associated with extensive rashes of the trunk and pruritus. With history of sintilimab use, clinical symptoms, and physical examination, TEN was diagnosed. Intravenous methylprednisolone and oral prednisone were administered until the patient totally recovered from the cutaneous toxicities caused by sintilimab. Monitoring of such rare but severe cutaneous toxicities is essential in patients who are treated with sintilimab.

KEYWORDS

immune-related adverse events (ir-AEs), neoadjuvant therapy, toxic epidermal necrolysis, non-small cell lung cancer, pulmonary tuberculosis

Introduction

With the wide spread use of immune check point inhibitors (ICIs) to treat the non-small cell lung cancer (NSCLC), immune-related adverse events (ir-AEs), known as toxicities associated with ICIs, during the treatment has been the focus of attention. Ir-AEs usually present as skin rash, thyroid dysfunction, hepatitis, colitis, and interstitial lung disease.

Cutaneous toxicities induced by ICIs account for approximately 33% all patients presenting with ir-AEs (1). Toxic epidermal necrolysis (TEN) is a cutaneous toxicity with detrimental effects on health. However, ICI-induced TEN has rarely been reported in literature. In this report, we describe a case of severe TEN that was induced by sintilimab after neoadjuvant therapy of a patient with advanced squamous cell-lung cancer and pulmonary tuberculosis.

Case report

A 59-year-old man was admitted to our hospital for the treatment of centrally located squamous cell-lung carcinoma and pulmonary tuberculosis. The computed tomography (CT) scans showed that the malignant lesion, located between the left upper and lower lobes, had invaded the left pulmonary artery (Figure 1). Pathological and etiological findings revealed a combined diagnosis of squamous cell-lung cancer (T3N0M0) and pulmonary tuberculosis. Anti-tuberculosis therapy, which including Isoniazid (300 mg per day), Rifampicin (450 mg per

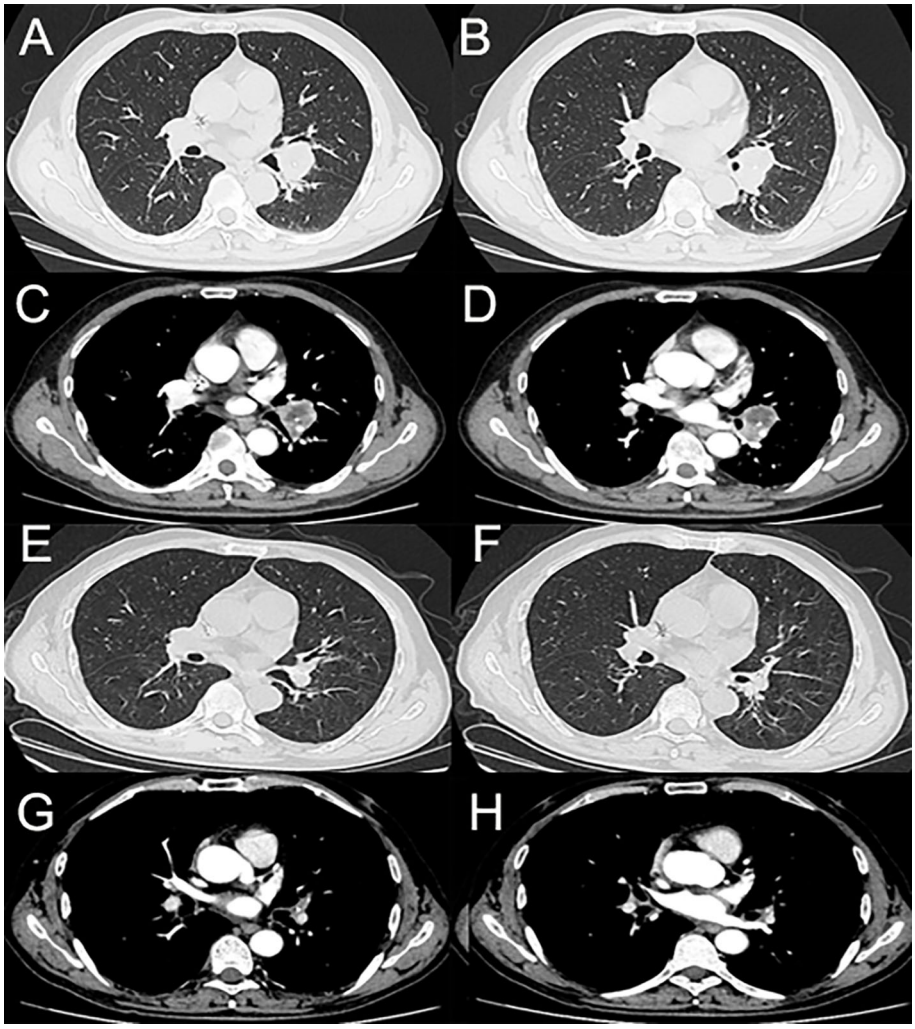

FIGURE 1
(A–D) The enhanced computed tomography (enhanced-CT) scans of the tumor before neoadjuvant therapy. **(E–H)** The enhanced-CT scans of the tumor after three cycles of neoadjuvant therapy.

day), Ethambutol (750 mg per day), and Pyrazinamide (1250 mg per day), without any adverse events, was administered to cure the pulmonary tuberculosis. Neoadjuvant therapy, including sintilimab and conventional chemotherapy, was administered to achieve a complete response (CR) or partial response (PR). After three cycles of neoadjuvant therapy, the patient underwent video-assisted thoracoscopic surgery (VATS) left lower lobectomy, because the malignant lesion had shrunk and the left pulmonary artery had been isolated from the tumor. No cutaneous toxicity was observed during the first three cycles of neoadjuvant therapy. According to the post-operative pathologic results, the neoadjuvant therapy resulted in a PR. Therefore, the sintilimab and conventional therapy were continued as post-operative adjuvant therapy.

Ten days after the post-operative adjuvant therapy, the patient suffered severe TEN, which rapidly progressed to cover >50% of the skin. TEN was associated with rashes of the trunk, pruritus, and a fever of >40 ℃. Massive maculopapular were observed in the chest and abdomen. Oral mucositis was also observed. The cutaneous lesions accounted for approximately 95% of the whole body surface (Figure 2). No pulmonary, gastrointestinal, or cardiac AEs were presented in this patient. Physical examinations showed the positive Nikolsky sign. Laboratory results showed that the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), C-reactive protein was 3.48, 217.39 and 53.28, respectively. The white blood cell was lower than normal because of chemotherapy. Due to financial constraints, no further skin biopsies were taken for pathological analysis. Because of the shortage of pathologic results, a dermatologist diagnosed TEN due to the history of sintilimab use, epidermal necrolysis, positive Nikolsky sign, high NLR and PLR, and so on. Exfoliative dermatitis, which was similar to TEN was suspected, but the Nikolsky sign was negative. Intravenous methylprednisolone (40 mg per day) was administered to this patient immediately. Additionally, prednisone (60 mg per day) was prescribed for out-patient use. Supportive management included wound care, nutritional supplementation, and analgesic use. Furthermore, Levofloxacin (500 mg per day) was administered to treat infections resulting from damage to the skin barrier. Four weeks after discharge from the hospital, we observed gradual healing of the epidermis with slight scars. Anti-tuberculosis therapy was continued for 4 months after diagnosis of TEN without any adverse events. The timeline of the treatment of the patient was presented in Figure 3.

Discussion

Due to the widespread use of ICIs in the treatment of advanced NSCLC, ir-AEs become the focus of attention because the incidence and motility rates of ICI-induced severe ir-AEs were 4% and 0.34%, respectively (2). Therapeutic doses of ICIs and platinum-based chemotherapy could be used in the neoadjuvant therapy for NSCLC (3, 4). ICIs are relatively safe for patients with lung cancer and comorbid tuberculosis; however, associated ir-AEs should be monitored cautiously (5). In this case, under the concomitant administration of anti-tuberculosis therapy, the tumor shrunk in size after neoadjuvant therapy. Thereafter, the patient underwent lobectomy safely without any adverse events.

According to a study in a tertiary hospital, the incidence of sintilimab-induced ir-AEs was 29.03%, which was lower than that caused by nivolumab, pembrolizumab, or camrelizumab (6). The most common symptoms of ir-AEs were nausea and vomiting but not cutaneous toxicities (6). Cutaneous toxicities, including alopecia, pruritus and rash, are prevalent in ICI-induced ir-AEs during and/or after the treatment of anti-NSCLC (7, 8). Few studies have reported severe dermatitis events, such as Stevens-Johnson Syndrome/Toxic epidermal necrolysis (SJS/TEN) (9–11) and severe oral mucositis (12). However, no case of severe sintilimab-induced TEN has been reported. In this case, severe TEN was almost fatal. The algorithms for causality defined the sintilimab in the categories

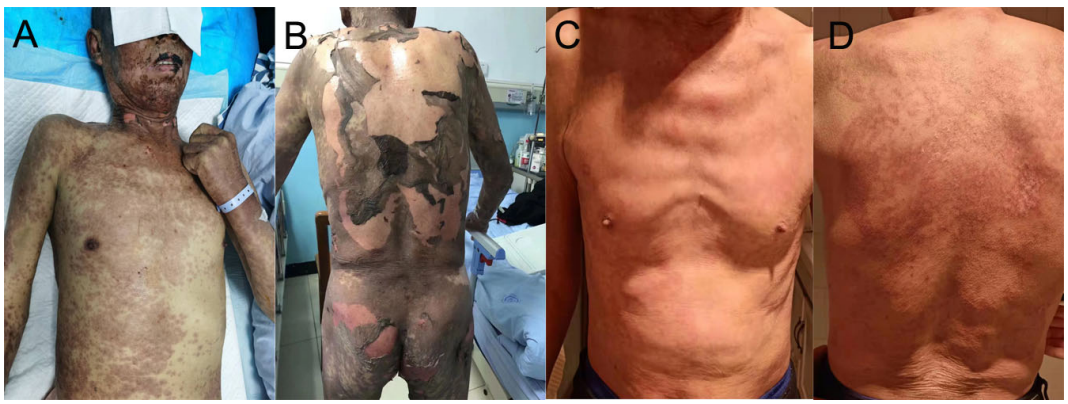

FIGURE 2
(A, B) TEN and rash induced by sintilimab in the patient with advanced NSCLC. **(C, D)** An image of the skin after recovery from severe TEN.

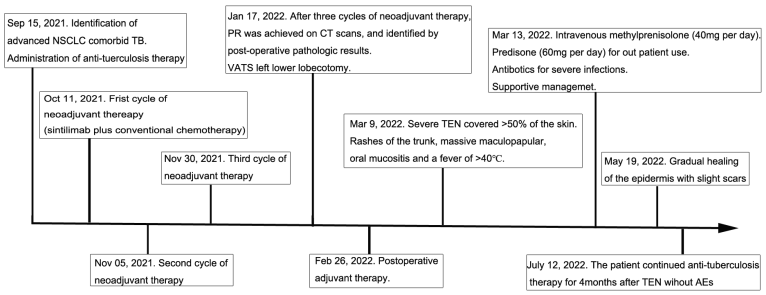

FIGURE 3
Anti-tuberculosis therapy: Isoniazid (300 mg per day), Rifampicin (450 mg per day), Ethambutol (750 mg per day), Pyrazinamide (1250 mg per day). Neoadjuvant therapy: Sintilimab (200 mg) plus platinum-based conventional chemotherapy (paclitaxel and cisplatin). PR, Partial response; TEN, Toxic epidermal necrolysis; AE, Adverse event.

"Probable" for this patient using the ALDEN causality scores (11, 13). The score of sintilimab was higher than that of the other medicines in the ALDEN causality score criterion. So we considered that the TEN was induced by sintilimab in this patient. Using the Common Terminology Criteria (CTC) for Adverse Events 5.0, grade 4 ir-AEs cutaneous toxicity was diagnosed.

Several predictive risk factors were associated with ICI-induced ir-AEs in the treatment of advanced NSCLC. According to previous reports, first-line treatment with ICIs and lung immune prognostic index (LIPI) were independent predictive risk factors for ir-AEs in patients with advanced NSCLC (14). Furthermore, concomitant chemotherapy use, high body mass index (BMI), and presence of epidermal growth factor receptor (EGFR) mutation were significant predictors for ir-AEs (15). BP180-specific IgG was associated with skin-adverse events in a histological study (16). Additionally, ICI combined with radiotherapy may cause severe skin-associated adverse events (17). For medicines that caused SJS/TEN more often than ICIs, no NSAIDs, anti-epileptic drugs, sulfa drugs, and allopurinol were used before and during the whole treatment. In this case, sintilimab was used as first-line treatment medication. However, the patient had a low BMI of 17.6 kg/m^2, which was not matched the previous risk factors. So debate was existed about the causality of the drug-induced clinical events. Hypersensitivity reactions of drugs may claim the reason of such reaction clinical symptoms (18). EGFR gene mutations and anaplastic lymphoma receptor factor (EGFR) were not evaluated because of the lower likelihood of those mutations in squamous cell carcinomas. Additionally, programmed cell death-1/programmed cell death-ligand 1 (PD-1/PD-L1) was not assessed due to patient's financial constraints. Since, TEN was related to ICI use as first-line treatment. Monitoring of the presence of severe cutaneous caused by ICIs was essential.

Appropriate treatment for TEN is crucial for patients with severe cutaneous toxicities, because ICI-induced TEN may be fatal during or after the treatment of advanced NSCLC. Methylprednisolone is the key regimen in the treatment of cutaneous toxicities, including rashes, bullous lesions, hypertrophic nodules, eruptive keratoacanthoma, papules, and plaques (19, 20). The patient recovered approximately 1 month post-discharge from the hospital after methylprednisolone was administered.

Limitations

Because skin biopsies were not conducted to this patient, diagnosis of TEN was mainly based on administration of sintilimab, physical examination, and laboratory results. It is the limitation of this manuscript.

Conclusion

Various forms of ICI-induced cutaneous toxicities during the treatment of advanced NSCLC have been reported. This case report was the first to describe severe TEN with associated extensive rashes of the trunk, limbs, and buccal mucosa 6 months after the initiation of sintilimab. Monitoring such rare and severe cutaneous toxicities is essential in patients treated with sintilimab. Further research is warranted for the incidence, pathophysiology, and mechanism of the ir-AEs associated with ICIs.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Ethical Review Committee for Research in The Public Health Clinical Center of Chengdu. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

GL, SG, and NW drafted the manuscript and obtained the image. XY supervised the manuscript writing. All the authors reviewed the manuscript and approved the final version.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Acknowledgments

The authors are grateful for the assistance provided by LiangShuang Jiang and other staff at Chengdu Public Health Clinical Center in preparing this manuscript.

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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frontiers | Frontiers in Immunology

TYPE Case Report
PUBLISHED 26 August 2022
DOI 10.3389/fimmu.2022.995121

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Immunology

RECEIVED 15 July 2022
ACCEPTED 08 August 2022
PUBLISHED 26 August 2022

CITATION
Ji H, Wen Z, Liu B, Chen H, Lin Q and Chen Z (2022) Sintilimab induced ICIAM in the treatment of advanced HCC: A case report and analysis of research progress.
Front. Immunol. 13:995121.
doi: 10.3389/fimmu.2022.995121

Sintilimab induced ICIAM in the treatment of advanced HCC: A case report and analysis of research progress

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Immune checkpoint inhibitor-associated adverse reactions (irAEs) are a clinical treatment issue that requires additional attention when ICIs have significant survival benefits in patients with advanced hepatocellular carcinoma (HCC). Among them, ICIs-associated myocarditis (ICIAM) is a kind of severe irAE with a high mortality rate (17%–50%). Despite its low incidence (PD1/PD-L1 related: 0.41%–0.8%), ICIAM can significantly disturb the decision making of therapeutic schemes and even the survival outcomes of patients. ICIAM induced by sintilimab has not been reported in any complete clinical studies yet and understanding the clinical characteristics involved may inform better practices for the management. Here, we reported a 78 y/o patient with advanced HCC, who experienced ICIAM induced by sintilimab within a short course from treatment onset and found that adequate baseline examination before the implementation of the therapeutic scheme, regular monitoring of myocardial enzymonram and cardiac imaging were measures for the early detection, while glucocorticoid pulse therapy is still the best choice with timely and sufficient application. Simultaneously, the combination of other immunosuppressants may lead to better results. New-predictive markers and examination methods are still required to facilitate the early detection.

KEYWORDS

hepatocellular carcinoma (HCC), programmed cell death receptor 1/ligand 1 (PD-1/PD-L1), ICI-associated myocarditis (ICIAM), sintilimab, immune checkpoint inhibitor-associated adverse reactions (irAEs)

Introduction

The application of immune checkpoint inhibitors (ICIs) has benefited the survival of patients with various kinds of advanced malignancies, such as non-small cell lung cancer (NCLC) and metastatic melanoma (1, 2). ICIs enhance the recognition of T cells by tumor cells by targeting the inhibition of cytotoxic T lymphocyte-associated protein 4 (CTLA-4)

or programmed cell death receptor 1 (PD-1)/ligand 1 (PD-L1) to alter the immune escape microenvironment of tumors. In recent years, breakthroughs have also been made in the systematic treatment of advanced hepatocellular carcinoma (HCC) with the application of immunotherapy, which has broken the state of monotherapy with only tyrosine kinase inhibitors (TKIs) (3). The "Standard for the diagnosis and treatment of primary liver cancer (National Health Commission of the PRC 2022, China)" recommends that HCC patients in China with liver cancer staging (CNLC) IIb, IIIa, and IIIb have a choice of combined systemic therapy (4). Sintilimab (Tyvyt®) is the second immune checkpoint inhibitor independently developed and marketed in China in 2018 and was approved for systemic treatment of advanced HCC (the fourth indication) in 2021. The effectiveness of sintilimab combined with lenvatinib has been reported in many clinical studies (5). However, the lack of reports on severe ICI-associated myocarditis (ICIAM) events induced by sintilimab during HCC treatment led us to report a 78-y/o patient with advanced HCC who experienced ICIAM induced by sintilimab within a short course from treatment onset here. The treatment regime is 21 d per cycle, and the adverse reaction occurred on the first day of the second cycle. The patient, who had no history of heart disease, experienced acute myocardial injury involving the conduction system with insidious and rapid progression but no obvious symptoms at the beginning. Within 4 days, the patient developed a significant conduction block accompanied by abnormalities of the myocardial enzymonram. The adverse reaction was controlled after temporary cardiac pacemaker implantation and a high dose of methylprednisolone (HDMP) combined with gamma globulin therapy.

Case presentation

The patient was a 78-year-old male, weighing 80 kg, with a history of hypertension. He was admitted to the hospital on 15 August 2017 for transhepatic arterial chemotherapy and embolization (TACE) treatment (surgical operation was rejected for the risks associated) due to HCC (CNLC Stage IIa/ Barcelona Clinic Liver Cancer, BCLC Stage B), and then received multiple TACE operations to control the tumor from 29 September 2017 to 9 September 2021. TACE was performed again due to the tumor progression on 1 March 2022, and a combined systemic therapy of sintilimab (200 mg Ivgtt 1/21 d) and lenvatinib (8 mg po 1/d) was planned. The therapeutic regimen was implemented on 3 March 2022. At that time, the baseline examination of the patient showed no obvious abnormalities in biochemical indicators, myocardial enzymonram, or electrocardiogram (ECG). Baseline monitoring was performed 1 week after the patient was safely discharged from the hospital after cycle 1, which showed no other changes except a mild liver function abnormality. When the patient planned to receive the second cycle of treatment on 24 March 2022, the baseline monitoring on admission showed abnormal elevation of creatine kinase (CK 1,330 IU/L) and creatine kinase-myocardial band (CK-MB 71.7 U/L). However, no abnormality was found in ECG or echocardiography. Therefore, the regimen was suspended for the consideration of safety, though the patient has not complained of any discomfort. After the cardiology consultation, it was suggested that additional measurements of troponin (cTnI), myoglobin (Mb), and BNP should be tested for further evaluation, and a 24-hour Holter examination should be performed for the differential diagnosis of non-ST elevation myocardial infarction (NSTEMI). On 28 March 2022, the CK (2,425 IU/L) and CK-MB (152.6 U/L) of the patient were further elevated with the abnormal of CTnI (0.86 ug/L) and Mb (>900 ug/L) at the same time. The ECG and the 24-hour Holter examination showed the complete right bundle branch block without significant ST-segment or T-wave abnormalities, and the patient still experienced no discomfort. So the diagnosis of the following: immune checkpoint inhibitor-associated adverse (irAE)-ICIAM G2 (Chinese Society of Clinical Oncology, CSCO, Management of immune checkpoint inhibitors-related toxicity 2021, China and CTCAE_4.03) was considered (6), and methylprednisolone was administered at 160 mg/d (2 mg/kg·d) for 3 days consecutively for treatment. After the condition of the patient improved, the dosage was reduced to 80 mg/d (1 mg/kg·d) from 1 April 2022. On 2 April 2022, the CTnI and NT-proBNP of the patient showed obvious rebounds. Simultaneously, the ECG suggested that the conduction block was aggravated and accompanied by the symptoms of fatigue and mild diarrhea. The patient was transferred to the cardiology hospital for further treatment immediately with a diagnosis of irAE-ICIAM G4 on 5 April 2022. On the day of transfer, a temporary cardiac pacemaker was implanted for the patient. On the third day after implantation, a 24-hour Holter examination was performed again, with the result still showing no abnormalities in ST-segment or T-wave. At the same time, the glucocorticoid therapy was adjusted to a pulse dose of methylprednisolone 1 g/d combined with gamma globulin 20 g/d for 3 consecutive days. The cardiac indicators and clinical symptoms of the patient improved significantly, with the ECG showing a turn back from III degree atrioventricular block to autonomic rhythm and the echocardiography showing no obvious abnormalities. Then, the standardized reduction of methylprednisolone was implemented (Figures 1–3).

Discussion

ICIAM is a rare kind of irAEs which can manifest as explosive myocarditis, pericarditis, conduction block, heart failure, myocardial fibrosis, or acute coronary syndrome (ACS) and has a high mortality rate (7).

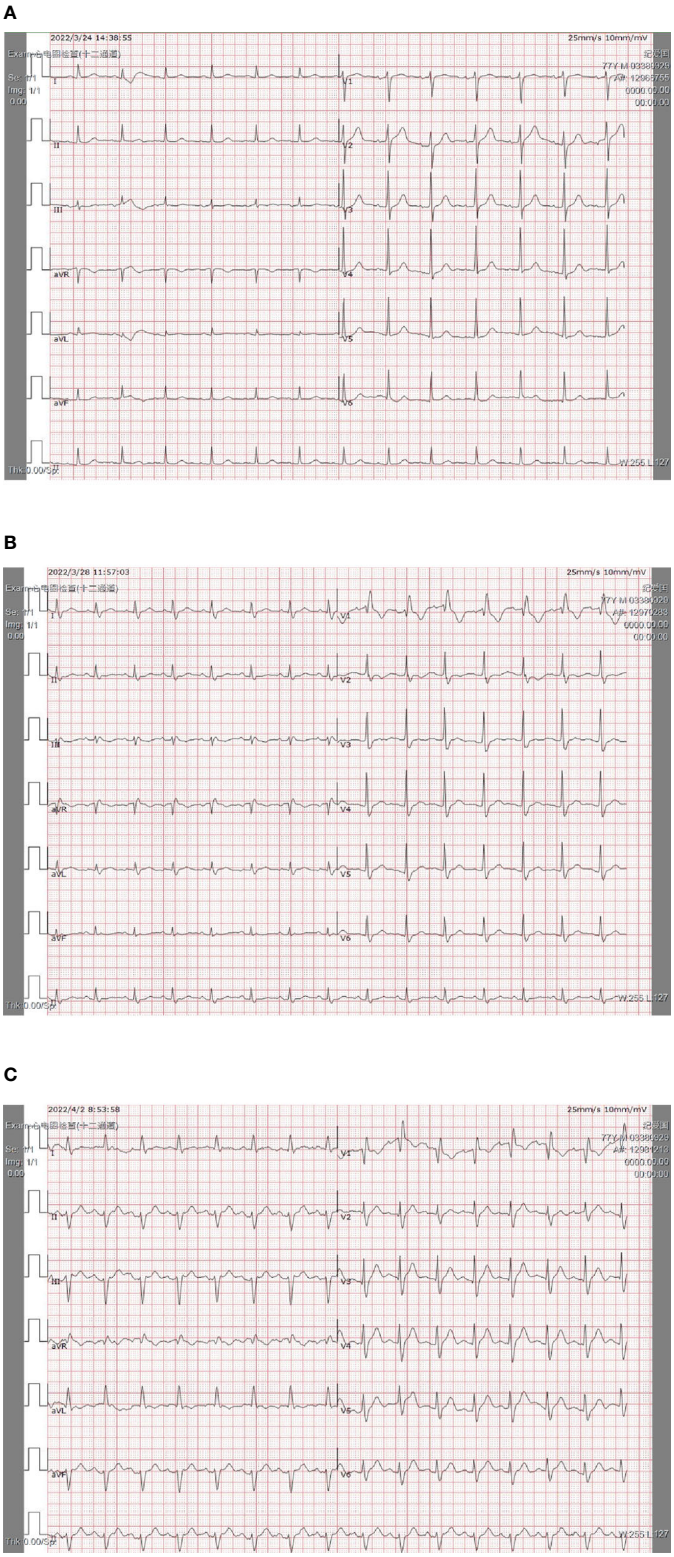

FIGURE 1
The rapid progress of the ECG of the patient's within 4 days. **(A)** PI and II were upright; Pav was inverted. QRS widened equal to or longer than 0.12 s; V1rsR; QRS terminal in each lead was blunt and ST-T was changed secondary. **(B)** PI and II were upright; Pav was inverted; QRS was widened equal to or longer than 0.12 s; V1 rsR; II, III, aVF were changed to rS type; S III >S II; I, aVL leads were qR type; R avL >R I; QRS electrical axis was left biased. **(C)** Sinus heart rate; high atrioventricular block; abnormal Q-waves appeared in V2–V3 leads.

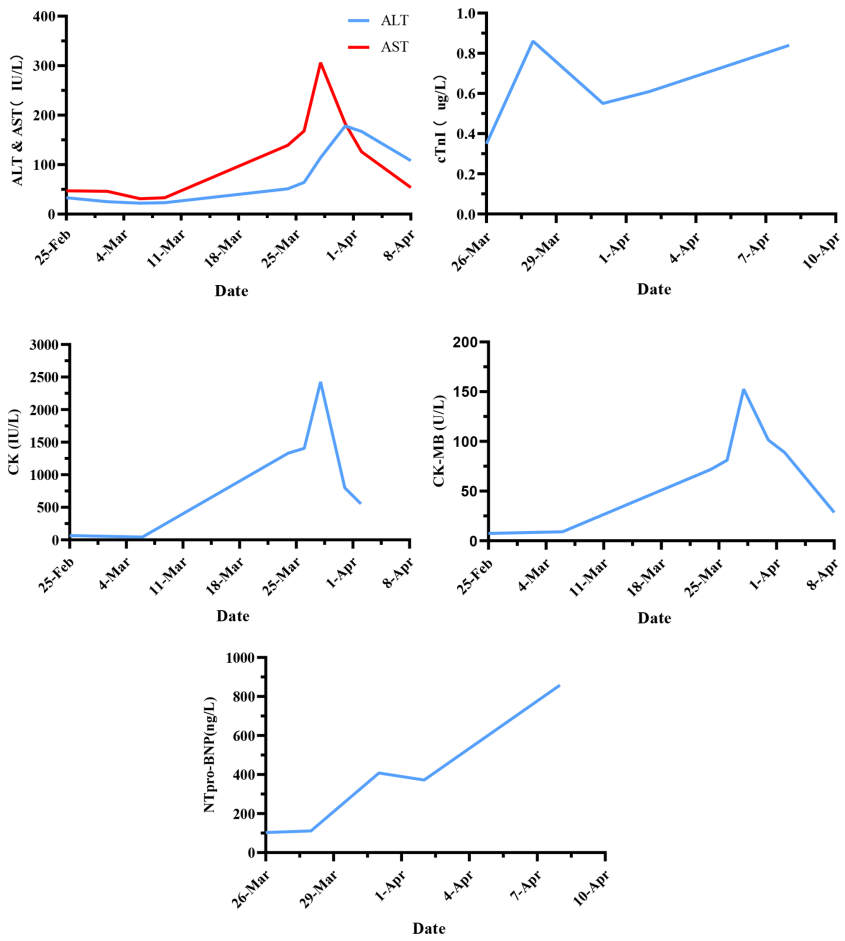

FIGURE 2
The changes in serum enzyme levels during the treatment (x-axis = date, y-axis = units symbols).

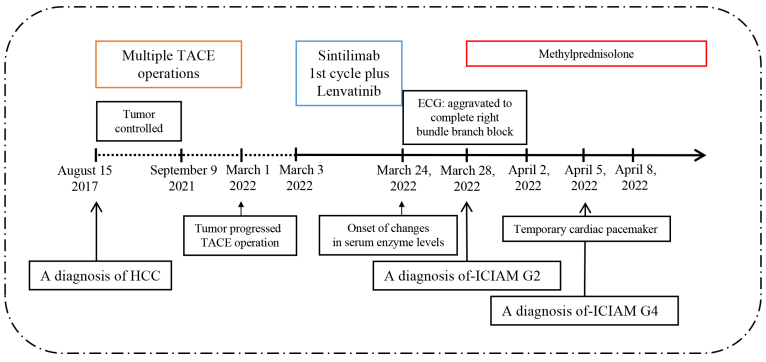

FIGURE 3
Timeline of disease diagnosis and treatment.

In several reports based on the World Health Organization (WHO) adverse drug reaction database (vigibase), the incidence of ICIAM is about 1.3%–5.8%, and the median time from the onset of ICI treatment to the occurrence of ICIAM is about 27–57 d, of which the incidence of ICIAM caused by PD-1/PD-L1 is about 0.41%–0.8%. In a retrospective analysis of 101 ICIAM cases, Moslehi et al. found that 64% of the patients experienced cardiotoxicity during the first or second cycle of ICI treatment, of which 76% occurred within the first 6 weeks from treatment onset, and 58 of the 101 patients were treated with PD-1 monotherapy (8–11). Another observational study from Jain et al. showed that conduction dysfunction accounted for 1.16% of ICIAM patients (12). The ORIENT-1 (NCT03114683) trial evaluated the safety and efficacy of sintilimab monotherapy among patients with relapsed or refractory classical Hodgkin's lymphoma who had received at least second-line chemotherapy. One patient developed ICIAM (G3) and was forced to stop treatment. In 2019, Rong Peipei et al. reported a patient with thymoma who received sintilimab combined chemotherapy and developed ICIAM rapidly. The time of occurrence was only 13 days after the treatment onset, which may be the first case report in clinical of sintilimab related ICIAM in China. Huang et al. reported in a study that 48 patients with various advanced tumors were treated with sintilimab, and one of them developed ICIAM (G1) (13). Huan Bi et al. reported a case with lung squamous cell carcinoma (SCC) that developed ICIAM within 6 days plus three cycles after receiving sintilimab treatment (21 days/cycle) (14). Chen and Liang et al. reported the case of ICIAM in a chordoma patient who received sintilimab plus anlotinib treatment respectively (15, 16). The retrospective analysis of cases from 12 cancer treatment centers in China by Wang et al. showed that the incidence of ICIAM in China was about 1.05%, the median time of occurrence was 38 d, and 81.2% occurred in the first or second cycle of ICIs therapy with a high mortality rate. Among them, the incidence of ICIAM caused by PD-1/PD-L1 was about 0.6% (17).

At present, the time definition of irAEs can be divided into early onset (median onset time ≤2 months) and late onset (median onset time >2 months). For ICIAM, Salem et al. found that the incidence of ICIAM decreased significantly after 3 months of flat treatment (9). Considering the current situation, it seems necessary to adopt a separate name and time definition for the serious and rapid-progressing style of irAEs, including ICIAM, and corresponding inclusion criteria should be formulated, so as to facilitate the accumulation of relevant cases and coping experience in each clinical center.

Before the implementation of ICIs treatment, the assessment of the baseline status of the patients is so important that for HCC patients the assessment should be very carefully as their liver function may fluctuate abnormally due to basic diseases (liver cirrhosis), tumor progression or other treatment interventions. In particular, it is not easy to determine the primary injured organs only based on the fluctuation of AST or CK in peripheral blood samples from an HCC patient. If there are no obvious clinical manifestations, the significant increase of CK or CK-MB compared to baseline data requires clinicians to be highly vigilant against the early occurrence of ICIAM. The early detection of ICIAM also requires attention to capture non-specific clinical manifestations in combination with the monitoring of ECG, myocardial enzymonram, echocardiography, or cardiac magnetic resonance. The retrospective study from Mahmood et al. showed that the abnormal rate of troponin, pro-BNP, and ECG in patients with ICIAM was 94%, 66%, and 89%, respectively, while the variation rate of left ventricular ejection fraction was only 49% (8). How to detect cardiac injury before the dysfunction of the left ventricular? A single center study by Zhao et al. showed that the global longitudinal strain (GLS) reduction may be an independent risk factor for ICIAM, which could be assessed early by using cardiovascular magnetic resonance feature tracing (CMR-FT) technology and could indicate cardiac injury prior to the onset of severe left ventricular dysfunction (18). Yu et al. found that the right ventricular systolic function often declined after PD-1/PD-L1 inhibitor treatment, and the decline mostly occurred during 21–42 d. This change can be detected early by using the two-dimensional speck tracing technique of echocardiography on the plane displacement of the tricuspid annulus during systole. In terms of research progress in predicting markers, studies have shown that soluble growth stimulating gene 2 protein (sST2) is a good marker of explosive myocarditis (19), which may be used to predict the prognosis of patients with ICIAM. In a meta-analysis of biomarkers for the detection of cardiac function disorders related to tumor therapy by Xiao et al., it was shown that the C-reactive protein (CRP), in addition to CTn and BNP, can also be an applicable biomarker for the occurrence of ICIAM (20). The upregulation of the CXC chemokine family, which is related to T-cell activation in the treatment of ICIs, may also suggest the occurrence of ICIAM (21, 22). Interestingly, John et al. reported three cases of ICIAM patients with diplopia and ptosis as the first clinical symptoms and suggested that clinicians should be alert to the possibility of ICIAM when patients appeared to have ocular symptoms (23). Presently, the "Chinese expert consensus on the surveillance and management of immune checkpoint inhibitor-related myocarditis" (2020 version) has recommended the classification and monitoring methods of ICIAM, which has an important reference value (24).

The first choice for coping with ICIAM is still impulse therapy with glucocorticoids. Mahmood et al. found that the initial high-dose of glucocorticoids would be more effective for patients, which can reduce the incidence of major adverse cardiac events (MACE) (8). Ma et al. reported eight cancer patients, including one HCC, who developed severe ocular symptoms during ICI treatment. These symptoms were controlled by systemic or topical glucocorticoid therapy, which

indicates that adequate treatment of glucocorticoids also plays a key part in the treatment of other types of irAEs (25). However, pretreatment of irAEs by using glucocorticoids is still not recommended because of the potential reduction in anti-tumor efficacy from ICIs, unless the patient has specific indications (e.g., infusion response or concurrent chemotherapy). If the condition of patients with mild myocarditis still deteriorates to a severe or critical type after receiving a routine dose, such as this patient, the glucocorticoids should be adjusted to the pulse dose without delay. In addition to glucocorticoids, there is no relevant research suggesting a better combination scheme. The selection of additional drugs may be based on the drug availability of medical institutions and the changes in the course of ICIAM. Many guidelines have recommended that if the condition of patients with severe myocarditis has not improved after receiving a pulse dose of glucocorticoids for 24 h, clinicians should apply one to two additional drugs jointly or sequentially, the drug choices including intravenous immunogloblin (IVIG), anti-thymocyte globulin (ATG), mycophenolate mofetil, tacrolimus, or infliximab (dose >5 mg/kg is forbidden for moderate to severe heart failure) (26, 27).

As a new immunotherapeutic drug for advanced HCC, sintilimab is being widely used in domestic tumor centers now due to its good efficacy, easy accessibility, and economic advantages. However, irAEs caused by sintilimab, especially ICIAM, are not completely controllable. Regular monitoring in the process of treatment for early diagnosis and the more aggressive application of glucocorticoids are preferred methods for coping with sintilimab-related ICIAM. Meanwhile, further experience needs to be accumulated from more clinical cases.

Data availability statement

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

Ethics statement

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

Author contributions

HJ and ZW contributed equally to this work. ZW provided case information and contributed to manuscript revision. HJ managed data analysis and drafted the manuscript. BL, HC, and QL performed the clinical management of the patient. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Key Laboratory of Biliary Tract Diseases of Xiamen and the Xiamen Medical and Health Guidance Project in 2021 (Approval number: 3502z20214zd1187).

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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frontiers | Frontiers in Immunology

TYPE Case Report
PUBLISHED 31 August 2022
DOI 10.3389/fimmu.2022.994064

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Immunology

RECEIVED 14 July 2022
ACCEPTED 08 August 2022
PUBLISHED 31 August 2022

CITATION
Lai K-C, Hsiao Y-H and Chen S-C (2022) Pulse corticosteroid therapy in the treatment of steroid-refractory immune checkpoint inhibitor-related pneumonitis: Case report and review. *Front. Immunol.* 13:994064. doi: 10.3389/fimmu.2022.994064

Pulse corticosteroid therapy in the treatment of steroid-refractory immune checkpoint inhibitor-related pneumonitis: Case report and review

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Immune checkpoint inhibitors (ICIs) have demonstrated promising therapeutic outcomes in treating a variety of malignancies, but immune-related adverse events (irAE) may develop. Among all the irAE, immune-related pneumonitis was relatively common and life-threatening. High-dose corticosteroid was recommended for the initial management, but a part of patients developed steroid-refractory pneumonitis. Other immunosuppressants were recommended, but the optimal treatment is still controversial. Here, we report two cases of steroid-refractory immune-related pneumonitis who were successfully treated with pulse corticosteroid therapy. Case 1 was hepatocellular carcinoma treated with nivolumab for 5 months. She developed acute respiratory distress syndrome due to grade 4 immune-related pneumonitis that was refractory to intravenous methylprednisolone 2 mg/kg/day treatment. Methylprednisolone 500 mg for 3 days followed by 2 mg/kg/day steroid as maintenance therapy was given. Subsequently, her pneumonitis was regressed, and the endotracheal tube was successfully removed on day 9 after the start of pulse therapy. Case 2 presented with grade 4 immune-related pneumonitis in spite the use of methylprednisolone 1 mg/kg for his skin rash. Pulse corticosteroid therapy was prescribed, then his pneumonitis was completely regressed on day 12. In this report, we demonstrated the potential role of pulse corticosteroid therapy for steroid-refractory pneumonitis.

KEYWORDS

Pulse corticosteroid therapy, steroid-refractory, immune checkpoint inhibitors, pneumonitis, immune-related adverse event, nivolumab

Abbreviations: ICIs, immune checkpoint inhibitors; irAE, immune-related adverse events; MMF, mycophenolate mofetil; HCC, hepatocellular carcinoma; TACE, trans-arterial chemoembolization; CNI, calcineurin inhibitor.

Introduction

In recent years, immune checkpoint inhibitors (ICIs) including anti-PD-1/PD-L1 and anti-CTLA-4 have played an important role in the treatment of various types of cancer. ICIs augment antitumor immunity by enhancing priming of CD4 T cells and cytotoxic function of CD8 T cells, which demonstrated a durable response in a part of cancer patients. However, with the activation of the immune system, ICIs also increased the risk of autoimmune toxic effects, termed immune-related adverse events (irAE). The common sites develop irAE including skin, lung, liver, and endocrine system.

Among all the irAE, immune-related pneumonitis is relatively common and life-threatening. The incidence of immune-related pneumonitis is about 5%, and the mortality rate of grade 3–4 immune-related pneumonitis is approximately 25%. The time to develop is approximately 9 days to19.2 months after the start of ICI therapy. Immune-related pneumonitis has various clinical manifestations, ranging from no symptoms, fever, shortness of breath, and even to respiratory failure and death. The immune-related pneumonitis is a diagnosis of exclusion. Pulmonary edema, infection, radiation pneumonitis, and cancer progression should be considered in the differential diagnosis. The radiological features of pneumonitis in chest computed tomography imaging could be various, including ground glass opacities, cryptogenic organizing pneumonia pattern, interstitial change pattern, consolidation, and traction bronchiectasis. Current guidelines recommend high-dose (1–4 mg/kg) prednisolone/equivalent corticosteroids for the initial management of immune-related pneumonitis. The efficacy of high-dose corticosteroid therapy reached approximately 70%–80% of response rate (1).

In some patients, pneumonitis could not be resolved despite high-dose corticosteroids for 48 to 72 h, which is known as steroid-refractory irAE. According to the current guidelines, other immunosuppressive agents, such as TNF-α inhibitor, intravenous immunoglobulin, cyclosporine, mycophenolate mofetil (MMF), cyclophosphamide, and tocilizumab, should be considered for severe or steroid-refractory irAE. Balaji et al. reported that 12 patients with steroid-refractory pneumonitis were treated with a TNF-α inhibitor (infliximab), intravenous immunoglobulin, or combined therapy, but the death rate was up to 75% (2). Beattie et al. described 26 patients with steroid-refractory pneumonitis treated with infliximab, MMF, or both, but only 10 patients (38.5%) had clinical improvement (3). Therefore, the optimal treatment of steroid-refractory pneumonitis is still controversial.

Here, we firstly reported two cases of steroid-refractory pneumonitis who were treated with pulse corticosteroid successfully.

Case report

Case 1

A 77-year-old woman had a medical history of hypertension and hepatitis B. She had never drunk alcohol, smoked tobacco, or taken illicit drugs. There was no previous medical history of lung disease and autoimmune disease. She was diagnosed with stage IB hepatocellular carcinoma (HCC) and then underwent resection in 2019. Due to repeated recurrence, she received several times of trans-arterial chemoembolization. Lenvatinib was prescribed but with poor response. Nivolumab was given for second-line treatment with the best response of stable disease. After 5 months of treatment, she complained of progressive exertional dyspnea. The oxygen saturation was down to 93%, and arterial blood gas analysis revealed paO_2 58 mmHg while the patient was breathing ambient air. Physical examination revealed bilateral rale breathing sounds. The electrocardiography disclosed a sinus rhythm without significant ST segment or T-wave change. The laboratory data disclosed WBC 4,900/μl, neutrophil-to-lymphocyte ratio 3.78, CRP 1.46 mg/dl, troponin I within the normal reference range, NT-proBNP 1,494 pg/ml, and d-dimer 5.6 μg/ml. Chest computed tomography demonstrated bilateral diffuse ground grass opacities but no evidence of pulmonary embolism. Other than that, irAE involving other organs was not observed. The impression was grade 3 immune-checkpoint inhibitor-related pneumonitis so that intravenous methylprednisolone 2 mg/kg/day was given. However, she presented with progressive hypoxia with a PaO_2/FiO_2 ratio of 135 which met the Berlin criteria of acute respiratory distress syndrome. Due to respiratory failure, she received endotracheal intubation with mechanical ventilation support. Repeat chest CT showed progressive bilateral crazy paving and consolidation. After consulting the pulmonologist, the patient was treated with intravenous pulse methylprednisolone 500 mg for 3 days followed by 2 mg/kg/day of steroid as maintenance therapy. In the following days, hypoxemia was rapidly improved (PaO_2/FiO_2 ratio 410) and chest X-ray image showed regression of bilateral lung infiltration. The endotracheal tube was successfully removed on day 9 after the start of pulse therapy, and she was discharged within 14 days (Figure 1A).

Case 2

A 44-year-old man had a medical history of stage IV chronic kidney disease. He did not smoke tobacco nor drink alcohol. No previous medical history of lung disease and autoimmune disease was documented. He was diagnosed with metastatic HCC in in June 2021 and received lenvatinib as first-line target therapy with poor response. Subsequently, nivolumab was prescribed as the second-line therapy. However, 10 h after nivolumab infusion, the patient experienced generalized pruritus and fever. The physical examination revealed a body temperature of 38.5°C and disseminated maculopapular skin rashes. There was no significant change in complete blood count/differential count and biochemistry data. Due to suspicion of intraabdominal infection, empirical antibiotics with ceftazidime was given. Grade 2 checkpoint inhibitor

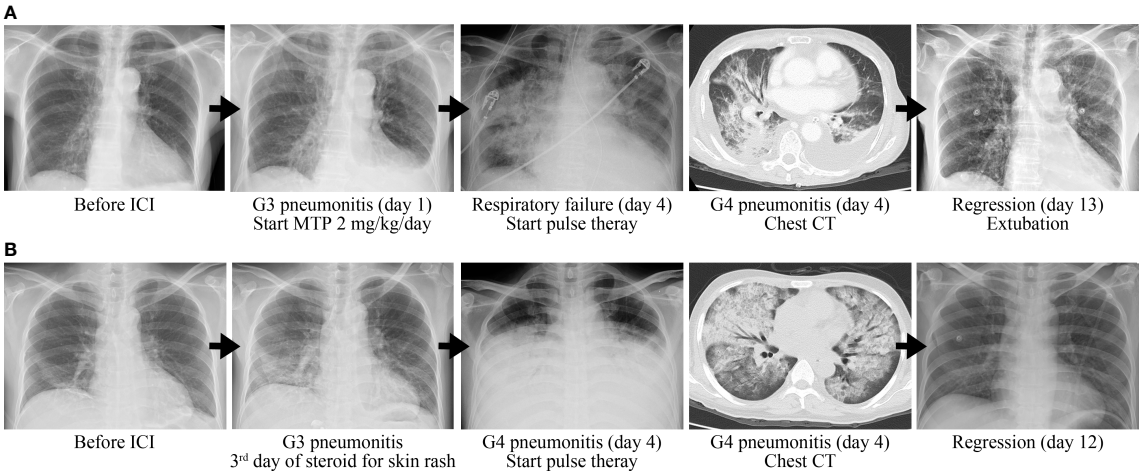

FIGURE 1
Immune-related pneumonitis. Case 1 was presented in **(A)**, and case 2 in **(B)**.

immune-related skin toxicity was also impressed, so intravenous methylprednisolone 1 mg/kg/day was prescribed. Three days later, the fever and rash alleviated but the patient developed dry cough and progressed dyspnea. The oxygen saturation was down to 67%, and the PaO_2/FiO_2 ratio was 66.3 under a non-rebreathing mask. The laboratory data showed WBC 11,800/μl, neutrophil-to-lymphocyte ratio 27.16, and CRP 21.92 mg/dl. Chest CT showed bilateral, multifocal ground grass lesions and no evidence of pulmonary embolism. Grade 4 immune-related pneumonitis was impressed. Since pneumonitis is refractory to the dose of methylprednisolone 1 mg/kg/day for 3 days, pulse corticosteroid therapy (methylprednisolone 500 mg/day for 3 days) was prescribed followed by 2 mg/kg/day methylprednisolone and mycophenolate mofetil (1,000 mg twice a day) for maintenance. The pneumonitis was regressive, and hypoxemia was improved (PaO_2/FiO_2 ratio 437.5). The patient no longer needed oxygen therapy 8 days from the start of pulse corticosteroid therapy (Figure 1B).

Discussion

Corticosteroids were widely used immunosuppressive agents. They inhibit a broad spectrum of immune cells and the synthesis of numerous inflammatory cytokines. At present, there is no clear definition for pulse corticosteroid therapy. In general, doses above 250 mg prednisone or equivalent steroids for days are considered as pulse corticosteroid therapy. The pulse steroid therapy plays a role in life-threatening autoimmune diseases, such as Goodpasture disease, necrotizing glomerulonephritis, and severe lupus nephritis. The clinical use of pulse corticosteroid therapy is summarized in Table 1.

Pulse corticosteroid therapy could rapidly achieve an immunosuppressive effect and avoid long-term steroid exposure in autoimmune diseases. A prospective lupus nephritis study reported 50 patients with class III, IV, or V lupus nephritis receiving rituximab, MMF, and two additional doses of 500 mg intravenous methylprednisolone. After 1 year of follow-up, the complete remission rate was 52% (4). A randomized controlled trial reported treatment strategies for lupus nephritis, which compared the efficacy of the intravenous cyclophosphamide group and combination therapy group (calcineurin inhibitor, MMF, and glucocorticoid). All the patients received 500 mg daily intravenous methylprednisolone as pulse therapy for 3 successive days and 0.6 mg/kg oral prednisone as maintenance treatment. After 6 months, the overall response rate in both groups was higher than 60% (5). A systematic review demonstrated that in comparison to the oral steroid-treated or untreated group, intravenous pulse corticosteroid therapy did not increase the risk of adverse effects, including neuropsychiatric, metabolic effect, and infectious complications (12). Therefore, intravenous pulse corticosteroid therapy could be considered a safe option for life-threatening autoimmune diseases.

To our best knowledge, the use of pulse steroid therapy for irAE was only reported in a few case series and case reports. In a report with 11 immune-related encephalitis cases, pulse steroid therapy demonstrated a high neurological improvement rate of 90% (6). Therefore, pulse steroid therapy was recommended for the initial treatment of immune-related encephalitis in the guideline (1). A case–cohort study enrolled 14 patients who

TABLE 1 The role of pulse corticosteroid therapy.

Research	Disease	Cases	Treatment	Outcome
Autoimmune disease				
2020, Condon et al. (4)	Lupus nephritis	50	• Two doses of rituximab (1 g), MTP (500 mg) on days 1 and 15, and maintenance treatment of MMF	• 1 year of complete remission rate: 52%
2021, Mejía-Vilet et al. (5)	Lupus nephritis	362	• MTP 500 mg for 3 days	24-week overall response rate • Multitarget treatment group: 83.5%; • Cyclophosphamide group: 63%
Immune-related adverse events				
2021, Taliansky et al. (6)	Encephalitis	11	• Low-dose steroids: 1 patient • Pulse MTP 1 g/day for 5 days: 10 patients (1 patient: +plasma exchange, 1 patient: + plasma exchange and cyclophosphamide)	Neurological improvement rate: • Low-dose steroids: 0% (0/1) • Pulse therapy: 90% (9/10)
2017, Makarious et al. (7)	Myasthenia gravis	23	• Pulse MTP: 5 patients	• Mortality rate: 40%
2020, Manohar et al. (8)	Acute interstitial nephritis	14	• Prednisolone (0.5-1 mg/kg/day) for 7 patients • Pulse MTP therapy 250-9750 mg for 7 patients	2-month complete response rate • Prednisolone: 60% • Pulse MTP therapy: 71.4%
2021, Oleas et al. (9)	Acute interstitial nephritis	8	• Prednisone 1 mg/kg/day: 5 patients • Pulse MTP 250-500 mg for 3 days: 3 patients	• 3-month complete response rate: 87% • Pulse therapy: 66.7%
2017, Ozaki et al. (10)	Cystitis	1	• MTP 500 mg for 3 days	• Complete recovery
Steroid-refractory ICI-related pneumonitis				
2021, Balaji et al. (2)	Immune pneumonitis	12	• Infliximab, IVIG, or combination therapy	• Death rate: 67%
2021, Beattie et al. (3)	Immune pneumonitis	26	• TNF inhibitor, MMF, cyclophosphamide, or combination therapy	• Recovery rate: 38%
2020, Utsumi et al. (11)	Immune pneumonitis	1	• Pulse MTP 1,000 mg for 3 days, tacrolimus, cyclophosphamide 500 mg therapy for 1 day	• Recovery

MMF, mycophenolate mofetil; MTP, methylprednisolone; IVIG, intravenous immunoglobulin.

were tissue-proven or were clinically suspicious of immune-related acute interstitial nephritis. Some patients were treated with variable doses of pulse corticosteroid therapy from 250 to 9750 mg. The complete response rate was 71.4% which is higher than in those treated without pulse therapy (8). Another study described eight patients who were diagnosed with immune-related acute interstitial nephritis which was confirmed by pathology. Three patients underwent intravenous pulse corticosteroid therapy. Renal function was recovered in two of them (67%), but one patient developed chronic kidney disease (9). A clinical case report demonstrated a man with advanced-stage lung squamous cell carcinoma who experienced grade 3 cystitis caused by nivolumab. However, supportive care and symptomatic management could not alleviate the symptoms. After receiving consecutive intravenous 500-mg doses of methylprednisolone for 3 days and oral prednisolone as maintenance treatment, the cystitis was improved (10). Besides, in a study of immune-related myasthenia gravis, five patients treated with pulse steroid therapy resulted with a myasthenia gravis-relevant mortality of 40% [2020 Huang]. For immune-related pneumonitis, only a case report described a man with metastatic non-small cell lung cancer who experienced steroid-refractory pneumonitis with pulse corticosteroid therapy failure. Subsequently, the pneumonitis was improved with the combination of high-dose corticosteroids, tacrolimus, and cyclophosphamide pulse therapy (11). Taken together, the role of pulse corticosteroid therapy for the treatment of irAE is potential but still lacks strong evidence for pneumonitis.

Conclusion

The current recommendation of the steroid-refractory immune pneumonitis is still controversial. The optimal treatment strategy presents a great challenge to clinicians. While pulse corticosteroid therapy offers a cost-effective

treatment option, more data are needed to support the role of pulse corticosteroid for steroid-refractory pneumonitis.

Data availability statement

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

Ethics statement

This study was reviewed and approved by institutional review board of Taipei Veterans General Hospital (TPEVGH IRB No.: 2021-08-005AC). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

Analyzed the data: K-CL, Y-HH, S-CC. Contributed reagents/materials/analysis tools: Y-HH, S-CC. Contributed to the writing of the manuscript: K-CL, S-CC. All authors contributed to the article and approved the submitted version.

Funding

This study is supported by Taipei Veterans General Hospital (V111C-131).

Acknowledgments

The authors wish to acknowledge the support of Taipei Veterans General Hospital (V111C-131).

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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frontiers | Frontiers in Immunology

TYPE Case Report
PUBLISHED 02 September 2022
DOI 10.3389/fimmu.2022.1001823

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Immunology

RECEIVED 24 July 2022
ACCEPTED 22 August 2022
PUBLISHED 02 September 2022

CITATION
Li X, Ji L, Li X, Sun D and Yang W (2022) Application of artificial liver in immune-related liver injury induced by immune checkpoint inhibitor: Case reports and review of the literature. *Front. Immunol.* 13:1001823. doi: 10.3389/fimmu.2022.1001823

Application of artificial liver in immune-related liver injury induced by immune checkpoint inhibitor: Case reports and review of the literature

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The use of immune checkpoint inhibitors (ICIs) can improve survival of patients with malignant tumors, however, the ICI treatment is associated with unpredictable toxicity as immune-related adverse effects (irAEs). Here we report two cases of metastatic malignant gastrointestinal tumors where severe immune-mediated hepatotoxicity (IMH) developed, characterized by liver failure, after the ICI therapy. Through a strong immunosuppressive treatment and a non-biological artificial liver and supportive treatment, the liver function was restored in both cases, and the anti-tumor treatment effect was guaranteed. These results showed that the non-biological artificial liver could be capable of improve prognosis during the ICI therapy.

KEYWORDS

immune checkpoint inhibitors (ICIs), artificial liver, immune-related adverse events (irAEs), immune-mediated hepatotoxicity (IMH), immune

Background

Immune checkpoint inhibitors (ICIs) have been shown to be effective in prolonging the survival of patients with various cancers, and a variety of ICIs are currently being developed and applied as monotherapy or combination therapy. However, in clinical applications by modulating immune response, ICIs may be associated with irAEs, potentially affecting diverse organs, including liver. These irAEs have different degrees, and can sometimes be fatal and life-threatening. ICIs-induced immune-mediated hepatotoxicity (IMH) is often manifested as asymptomatic elevation of alanine

aminotransferase (ALT) and/or aspartate aminotransferase (AST), with or without increased bilirubin. It may also be accompanied by non-specific symptoms such as fever, fatigue, and decreased appetite. Elevated bilirubin can cause yellow skin and sclera, yellow urine, etc. However, in rare cases, IMH can even cause acute liver failure (1).

For the treatment of IMH, various guidelines recommend the use of glucocorticoids as the main treatment drugs. For patients without any contraindication to hormones, the active hormone therapy is recommended. For patients with rapid disease progression or liver failure, plasma exchange may be an optional treatment (2).

As one of the effective methods for the treatment of liver failure, the non-biological artificial liver removes various harmful substances from liver, supplements essential substances to liver, and improves the internal environment within liver. It can temporarily compensate a part of the function of failed liver and create physiological conditions for the regeneration of liver cells, and the recovery of liver function was conducted by an *in vitro* mechanical, physicochemical and biological device. Currently, the following modes are commonly used in clinical practice for liver failure: plasma exchange (PE), double filtration plasmapheresis (DFPP), double plasma molecular absorb system (DPMAS), and plasma diafiltration (PDF). For the present study, we treated two patients with liver failure induced by ICIs by application of the artificial liver, which effectively restored liver function in both cases. Our results showed a remarkable clinical effect by the application of artificial liver.

Case presentation

Case 1

A 54-year-old man was admitted to our hospital on August 11th, 2021 due to yellow staining of skin and sclera for 18 days and fever for 3 days. On April 22th, 2021, the color doppler ultrasound revealed pancreatic space-occupying, following this, relevant examinations were performed to confirm the diagnose of pancreatic head cancer, gastric wall invasion, and abdominal lymph node metastasis. From June 2 to June 23th, 2021, the systemic chemotherapy combined with immunotherapy was given to this patient, more specifically: nab-paclitaxel 200mg d1, 8+ Seggio Capsules 60mg bid d1-14, sintilimab 200mg d1/3w were injected for this combined treatment. His liver function was normal before treatment. On July 5th, 2021, the patient developed a scattered rash all over the body, which was diagnosed as immune-related dermatitis. This symptom of the patient was improved after a hormone therapy. On July 25th, 2021, fatigue, anorexia, and yellowing of the skin and sclera were observed in the patient. The patient had fever on August 8th, with a body temperature of up to 39°C and without cough, expectoration, dysuria, urgency, abdominal pain, and diarrhea. The antibiotic and glucocorticoid (2 mg/kg/d) treatment for the patient lasted 3 days, resulting in a disatisfactory treatment effect. Afterwards, the patient was admitted to the hospital for further diagnosis and treatment. He had no history of hypertension, diabetes, heart disease, blood transfusion, and drug allergy. Physical examination: the sclera and skin of the whole body were severe yellow staining, scattered erythema and papules, and no liver palm and spider nevus were observed.; the breath sounds of both lungs were clear, with the absence of wet or dry sounds; the heart sounded strong, with a regular rthythm of a heart rate of 68 beats/min; the abdomen was soft, with the absence of tenderness and rebound tenderness; the liver and spleen were not under the ribs; no percussion pain in the liver area was observed, and mobility dullness was negative; no edema was noted on either leg.

Laboratory examinations showed the following results: Whole blood cell analysis: WBC:11.2×109/L, NEUT#:8.68×109/L, PLT:43×109/L; Coagulation: PT:29.3s, INR:2.74, PTA:26%, APTT:35.7s; Blood biochemical examination: ALT:1736.4IU/L, AST:800IU/L, TBil:167μmol/L, DBil:98.8μmol/L, D/T:0.59, γ-GT:256.7IU/L, ALP:102.2IU/L; HBsAg, HBeAg, HBcAg, HBsAb, HBeAb, HBcAb, Hepatitis A antibody, Hepatitis C antibody and Hepatitis E antibody IgM were all negative. AFP: 5.8ng/mL; autoantibodies were negative; Immunoglobulin IgG10.8g/L, IgA 3.84g/L, IgM0.73g/L. Abdominal MRI (September 10th, 2021): cystic mass in the neck of the pancreas with nodules on the right wall. Liver puncture was not performed for the patient due to coagulation dysfunction. Final diagnosis: Immune-related liver injury induced by ICIs, grade 4, with a subacute liver failure, early. The diagnosis basis of this patient is shown in Table 1 (3).

Then, Sindilizumab was stopped immediately, intravenous magnesium isoglycyrrhizinate 200mg once a day, AdenosylMethionine 1g once a day and glucocorticoid (2mg/kg/d) were given. And on August 13th, 16th, and 18th, the single-level plasma exchange (PE) treatment was performed respecitively, and the dose of each treatment was the same, namely 2000ml fresh frozen plasma. Liver function and coagulation indicators were improved significantly after this treatment (see Figures 1A, B). Afterwards, sintilimab was discontinued, and systemic chemotherapy was used for 3 cycles, the liver function was normal during this period of treatment. Repeat abdominal MRI on December 9th: the size of cystic mass in the neck of the pancreas was smaller than that observed on September 10th, 2021 (see Figures 1C, D).

Case 2

A 64-year-old woman was admitted to our hospital on December 3th, 2021 due to the "diagnose of liver metastatic adenocarcinoma for more than 3 months and fatigue for 1

TABLE 1 Grading and management principles of immune-mediated liver injury induced by ICI.

Grading	Management
G1: Asymptomatic (AST or ALT >ULN to 3.0 × ULN and/or total bilirubin >ULN to 1.5 × ULN)	Continue ICPi with close monitoring; consider alternate etiologies. Consider monitoring labs 1-2 times weekly. Manage with supportive care for symptom control. Hold ICPi temporarily. Patients should be advised to stop unnecessary medications and any known hepatotoxic drugs. Temporarily hold other potentially hepatotoxic oncologic agents.
G2: Asymptomatic (AST or ALT> 3.0 to ≤ 5 × ULN and/or total bilirubin > 1.5 to ≤ 3x ULN)	For grade 2 hepatic toxicity, may administer steroid (0.5-1 mg/kg/d prednisone) or equivalent if no improvement is seen after 3-5 days. Increase frequency of monitoring to every 3 days. If inadequate improvement after 3 days, consider adding mycophenolate mofetil. May initiate steroid taper when symptoms improve to ≤ G1 and may resume ICPi treatment when steroid ≤ 10 mg/d. Taper over at least 1 month. Consider hepatology consult for G2 and above. May resume if recover to ≤ G1 on prednisone ≤ 10 mg/d. Follow G2 recommendations as listed, with the following additions for G3: Consider permanently discontinuing ICPi if asymptomatic; permanently discontinue if symptomatic. Immediately start steroid 1-2 mg/kg methylprednisolone or equivalents.
G3: AST or ALT 5-20 × ULN and/or total bilirubin 3-10 × ULN, OR symptomatic liver dysfunction; fibrosis by biopsy, compensated cirrhosis; and reactivation of chronic hepatitis	If steroid refractory, consider liver biopsy to rule out NASH, tumor, cholestatic variants, other drug-related hepatic inflammation, infection, or other autoimmune entity and consider adding azathioprine[b] or mycophenolate[c] if infectious cause is ruled out. Labs daily or every other day: consider inpatient monitoring for patients with AST/ALT > 8 × ULN and/or elevated total bilirubin 3 × > ULN. If no improvement is achieved with steroid or for patients on ICPi therapy combined with a novel agent, with standard CTX, or with targeted therapy, refer to hepatologist for further pathologic evaluation of hepatitis. Steroid taper can be attempted around 4-6 weeks when ≤ G1, re-escalate if needed, optimal duration unclear. Consider transfer to tertiary care facility if necessary.
G4: AST or ALT > 20 × ULN and/or total bilirubin > 10 × ULN OR decompensated liver function (eg, ascites, coagulopathy, encephalopathy and coma)	Follow G3 recommendations as listed, with the following additions for G4: Administer 2 mg/kg/d methylprednisolone equivalents

week". In early August 2021, the patient had been admitted to our hospital because of abdominal pain and low back pain. PET-CT (Positron emission tomography-computed tomography 2021/8/13): 1.Multiple hypermetabolic nodules and masses in the liver; low density shadow of portal vein, considering tumor thrombus; Multiple lymph node metastasis in mediastinum, hepatogastric space, hilar region and retroperitoneum; Multiple bone metastasis. 2.A hypermetabolic mass in the hilar region is considered to be malignant. Further pathological immunohistochemical (IHC) results showed that AE1/AE3 (+), Hepatocyte (partial +), AFP (-), GPC-3 (focal +), HSP70 (+), GS (-), Arginase-1 (focal +), IMP3 (diffuse +), CK7 (minority +), CK20 (+), CEA (-), CDX-2 (-), SATB2 (+), Villin (+), GATA-3 (-), TTF-1 (-), Napsin A (-), p53 (80% +). Ki67 (30% +). Based on the results of imaging examination and pathological immunohistochemistry, the patient was diagnosed as intrahepatic cholangiocarcinoma with multiple metastases after MDT multi-disciplinary consultation. cTNM staging: stage IV. From August 16th to November 8th, paclitaxel (albumin-bound) combined with Seggio and pembrolizumab was given to the patient for 5 cycles. Her liver function was normal before treatment. At the end of November 2021, the patient developed fatigue, anorexia, and abdominal distension, without the symptom of abdominal pain, fever, nausea, and vomiting. She was admitted to the hospital for further diagnosis and treatment. The patient has a history of hepatitis B, but no history of hypertension, diabetes, heart disease, blood transfusion, and drug allergy. Her physical examination revealed the following: The sclera and skin of the whole body were normal, no liver palm and spider nevus were seen; the breath sounds of both lungs were clear, with the absence of dry and wet sounds; the heart sounded strong, with a regular rhythm of a heart rate of 62 beats/min; the abdomen was soft, without tenderness and rebound tenderness; the liver was 4 transverse fingers below the right ribs, and the mobility dullness was negative; no edema was noted on either leg.

Laboratory examinations showed the following results: Whole blood cell analysis (2021-12-4): WBC:2.8×109/L, NEUT#:1.61×109/L, PLT:57×109/L; Coagulation (2021-12-4): PT:14.6s, INR:1.34, PTA:66%; Blood biochemical examination (2021-12-4): ALT:774IU/L, AST:1113.5IU/L, TBil:26.6μmol/L, DBil:13.5mol/L, γ-GT:160.6IU/L, ALP:229.5IU/L;ICI was stopped immediately, intravenous magnesium isoglycyrrhizinate 200mg, reduced glutathione 1.8g once a day to protect the liver.Other examination (2021-12-4): HBsAg(+)、HBeAg(+)、HBcAg(-)、HBsAb(-)、HBeAb(+)、HBcAb(+), HBV DNA:<100IU/mL, Hepatitis A antibody, Hepatitis C antibody and Hepatitis E antibody IgM were all negative.AFP:3.5ng/mL; autoantibodies were negative; Immunoglobulin IgG9.5g/L、IgA 2.58g/L、IgM1.2g/L. Liver biopsy was recommended, which was rejected

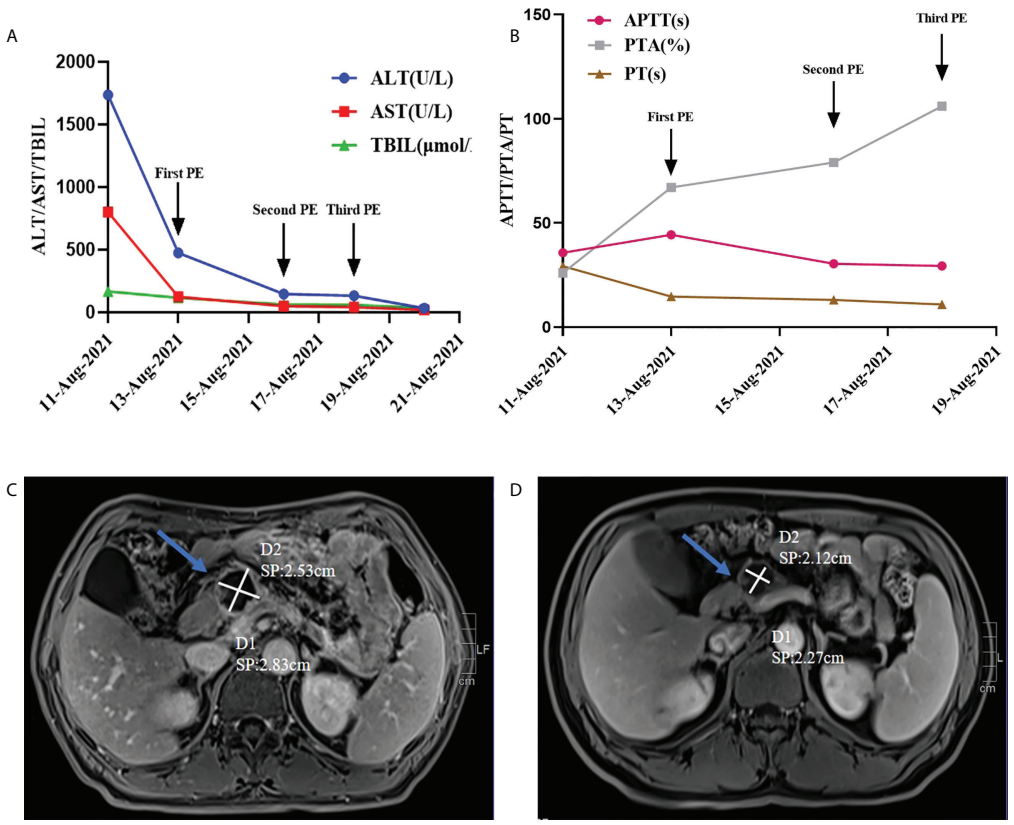

FIGURE 1
(A, B) Changes of various indicators before and after artificial liver treatment; **(C)** On September 10, 2021, the abdominal MRI of the pancreatic neck was about 2.5*3.04cm in size (indicated by the arrow); **(D)** On December 9, 2021, the abdominal MRI of the pancreatic neck occupies a space of about 2.16*2.37cm (indicated by the arrow).

by the family. (2021-12-10):ALT(228.4IU/L) and AST(749.3IU/L) were lower than before,TBil(119.3 umol/L) and γ-GT(188IU/L) were higher than before, and she gradually developed yellow skin and sclera, dark urine, extreme fatigue, abdominal distension, and nausea. Considering immune-mediated hepatotoxicity, glucocorticoid(1mg/Kg/d) was given (12/10-12/20), Combined with oral mycophenolate mofetil(MMF) 0.5g twice a day (12/18-12/20). Blood biochemical examination (2021-12-20): ALT: 842.6IU/L, AST: 1249.7IU/L, TBil: 249.4μmol/L, DBil: 93.8mol/L. Coagulation (2021-12-25): PT: 26.2s, INR: 2.34, PTA: 30%, APTT: 39.1s. Abdominal CT (2021-12-26): hepatic hilar space occupying and liver metastasis of the patient was improved compared with that in the previous examination. Liver cirrhosis, enlarged spleen, widened portal vein were observed in the patient during this period of examination. Final diagnosis: Immune-related liver injury induced by ICIs, grade 4. with a subacute liver failure at an early stage. Diagnosis was confirmed based on the same results as previous ones.

Subsequently, from December 22th to 24th, she was given a double plasma molecular adsorption therapy. On December 27th, she underwent single-plex plasma exchange, replacing 2000ml of fresh frozen plasma. On December 30th, double plasma molecular adsorption combined with half-volume plasma exchange therapy was given to her, and the amount of plasma exchanged was 1000ml (see Figure 3). After this treatment, her liver function returned to normal (see Figures 2A, B). Subsequently, the re-examination showed that her liver lesions had shrunk significantly (see Figures 3A–D).

Discussion

With the widespread use of ICIs, irAEs have increasingly attracted the attention of clinicians. Among them, IMH induced by ICIs can even lead to liver failure or death, and in severe cases it can threaten the life of patients. According to clinical reports, the rate of occurrence of ICIs-induced IMH is approx 3-10% (4–6). The pharmoco-vigilance database of World Health Organization (WHO) showed that among 613 fatal adverse events caused by ICIs this year, 20.2% (124/613) of patients died of IMH (7). These factors indicate that the risk of death caused by IMH should be paid more attention.

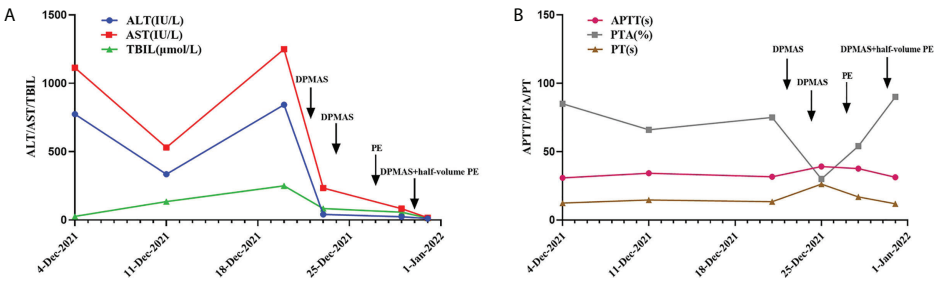

FIGURE 2
(A, B) Changes of various indicators before and after artificial liver treatment.

The mechanism of IMH occurrence is not fully understood. The liver has evolved high immune tolerance due to continuous exposure to foreign antigens, and blockade of immune checkpoints may lead to abnormal immune activation, affecting the liver of patients. At present, it has been found in human specimens and animal models that the occurrence of IMH is largely caused by the activation of CTL. $CD8^+$ T-predominant lymphocytic infiltration has been observed in both human and mouse livers treated with ICIs and elevated transaminases (8–10). In addition, various $CD4^+$ T helper cells and Treg cells are also involved in the liver injury of IMH (11, 12). It is speculated that the possible mechanism is that ICIs induce lymphocyte activation and mediate hepatocyte death by expressing death ligands, exocytosing granules or producing various cytokines such as IFN-γ and TNF (10). In addition, secondary activation of the innate immune system has also been found to be involved in immunosuppressive-induced liver injury (11). It has been reported that hepatocyte injury and cell death in

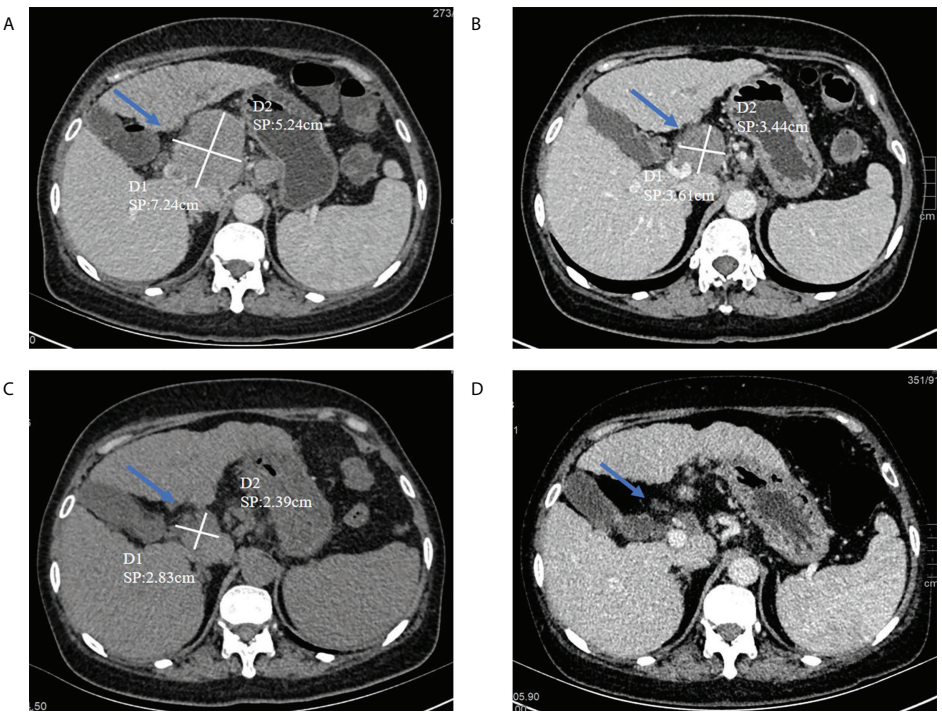

FIGURE 3
(A) On August 6, 2021, abdominal CT (before treatment) had a tumor in the hepatic hilum area, with a size of about 5.24*7.64cm (indicated by the arrow); **(B)** The tumor in the hepatic hilum area on September 25, 2021, about 3.44*3.61cm in size (indicated by the arrow); **(C)** On January 20, 2022, abdominal CT (after treatment) had a tumor in the hepatic hilum area, with a size of about 2.39*2.83cm(indicated by the arrow); **(D)** On Apirl 23, abdominal CT showed that the tumor had basically disappeare.

IMH may be caused by the crosstalk between innate and adaptive immunity (13). However, its specific mechanism has not been fully elucidated.

IMH has been reported to occur 6 to 12 weeks after the first ICI dose (14), but may also occur after longer periods of treatment. In this case 1, the patient developed multiple rashes all over the body after using ICIs for 12 days, which considered skin damage from ICIs. After hormonal treatment, the patient improved, with fatigue, anorexia, yellow skin, significantly elevated liver enzymes, and coagulation dysfunction in 53 days. And excluded viral hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease, autoimmune liver disease, cholestatic disease, genetic metabolic disease and other liver diseases. No liver tumor lesions or biliary obstruction were found by imaging examination, and the RUCAM scale judged causality with a score of 8 (highly likely). After ICIs treatment, the patient developed skin and liver damage successively, and multiple irAEs have been observed in clinical practice (15). Nearly half of IMH patients have other types of irAEs (4), so the combination of irAEs is more helpful for the diagnosis of IMH. In this case, the diagnosis of immune-related liver injury and hepatocyte injury induced by ICIs was established.

In case 2, the patient developed fatigue and anorexia after using ICIs for 14 weeks. Laboratory tests showed a significant increase in transaminase, followed by a rapid decline in transaminase, progressive deepening of jaundice, and abnormal coagulation (PTA decreased to 30%). Although combined with a history of hepatitis B, the long-term antiviral treatment effect is good, and the HBV DNA detection is within the normal range. Liver diseases such as alcoholic liver disease, non-alcoholic fatty liver disease, autoimmune liver disease, cholestatic disease, and genetic metabolic disease were excluded. Imaging examination of the liver tumor was smaller than before, and no biliary obstruction was found. So, the patient was diagnosed with immune-related liver injury induced by ICIs.

At present, major guidelines (16, 17) all use the liver toxicity grading standard (according to the level of transaminase and bilirubin) in the common terminologycriteria for adverse events (CTCAE) to guide the treatment of IMH. Glucocorticoids are the main treatment drugs recommended by the guidelines, which have the effect of suppressing the immune response. It can improve the immune damage of target organs, stabilize the lysosomal membrane, reduce the non-specificity of portal area and capillary bile ducts, prevent the production of antigen-antibody complexes through anti-endotoxin effect, prevent further damage to liver function, and gradually improve liver function. If there is no response to hormone therapy for 3 days, the addition of mycophenolate can be considered. The current guidelines do not make clear recommendations for third-line immunosuppressive therapy. Tacrolimus and antithymocyte globulin (16) can be added. Considering the issue of liver toxicity, infliximab is not recommended. Two patients had rapid disease progression after liver injury, and they were diagnosed with subacute liver failure. After adequate glucocorticoid, liver protection, jaundice, nutritional support and other comprehensive treatment, the effect is not good.

Non-biological artificial liver supportive therapy has become an important treatment method for liver failure and has been widely used in the clinical applications, and its clinical efficacy has been demonstrated in different studies (18). It was reported that plasma exchange was used to treat acute liver failure caused by yellow phosphorus poisoning, the overall survival rate of patients reached 79.06% (19).Despite low overall incidence rate of IMH, fulminant liver failure(0.1% to 0.2%) can occur sporadically (20). Artificial liver therapy such as plasma exchange can remove a large amount of toxins, simultaneously infuse fresh normal plasma and supply diverse nutritious factors such as albumin and coagulation factors, which effectively compensates the liver detoxification and synthesis function and provides optimal internal environment for liver repair. During and/or after artificial liver therapy, the liver enzymes can return to a normal status, the level of bilirubin decreases, and the coagulation function is improved. These results showed that this therapy is effective, which not only saves the patient's life, but also form a clinical basis for follow-up therapeutic intervention.

In the present study, the patient in case 1 experienced recovered liver function following artificial liver therapy and continued anti-tumor treatment. The re-examination showed that the volume of tumor lesion was shrunk by more than 30% according to the evaluation criteria for solid tumors (RECIST 1.1). The overall treatment result was evaluated as partial remission, and the patient successfully completed conversion therapy and is currently undergoing a surgical treatment. The patient in case 2 was diagnosed as advanced hepatic malignant tumor. After artificial liver therapy, the liver function of this patient was improved. The re-examination result showed that the shrunk volume of tumor lesion in this patient was more than 80%. The overall evaluation of treatment was partial remission. Unfortunately, the results of liver biopsy were not obtained due to the significant abnormal blood coagulation and high risk of liver puncture bleeding in case 1, and the refusal of the family members of case 2, which needs to be further improved.

Conclusion

In summary, liver failure caused by immune checkpoint inhibitors can be treated with artificial liver therapy that can significantly improve the liver function and ensure therapeutic effectiveness and safety during anti-tumor treatment.

Data availability statement

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

Ethics statement

Written informed consent for the publication of any identifiable images or data was obtained from the patient.

Author contributions

XuL, LJ, XiL, DS and WY: study concept and design the overall study. XuL, LJ and XL: analyzed and interpreted the patient data. XL and WY: drafted the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This case report was supported by grants from the National Natural Science Foundation of China (No. 82002619), the Key Medical Research Projects of Shanxi Province (No. 2020XM55), and the Talent Introduction Scientific Research Start-up Fund of Shanxi Bethune Hospital (No. 2020RC006).

Acknowledgments

We thank the patients for their permission to publish this case report and for their friendly cooperation with providing necessary data.

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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frontiers | Frontiers in Immunology

TYPE Case Report
PUBLISHED 15 September 2022
DOI 10.3389/fimmu.2022.998516

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to
Cancer Immunity
and Immunotherapy,
a section of the journal
Frontiers in Immunology

RECEIVED 20 July 2022
ACCEPTED 01 September 2022
PUBLISHED 15 September 2022

CITATION
Wang Y, Wang Y, Yu J and Meng X
(2022) Case report: Pneumonia with
clinical symptoms precedes imaging
evidence after immune checkpoint
inhibitors combined with radiotherapy
in lung squamous cell cancer.
Front. Immunol. 13:998516.
doi: 10.3389/fimmu.2022.998516

Case report: Pneumonia with clinical symptoms precedes imaging evidence after immune checkpoint inhibitors combined with radiotherapy in lung squamous cell cancer

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Immune-checkpoint inhibitors (ICI) targeting programmed cell death 1 (PD-1) and its ligand 1 (PD-L1) have quickly changed the treatment landscape in advanced non-small cell lung cancer. However, any patient treated with an immune checkpoint inhibitor is at risk for immune-related adverse events (irAEs). Checkpoint inhibitor pneumonitis (CIP) is a rare but potentially severe pulmonary toxicity of immunotherapy. Since the imaging features and symptoms are not specific, the diagnosis of CIP is challenging. In addition, CIP may mimic other lung diseases. Due to these characteristics, proper patient management may be delayed. So, a comprehensive understanding of imaging features is essential for a prompt detection and correct management of these drug-induced lung diseases. We presented a patient with lung squamous cell cancer who has clinical symptoms preceding imaging evidence of pneumonitis after immunotherapy and radiotherapy. We also discussed the safety of immunotherapy, the complexity and management of immune pneumonitis.

KEYWORDS

immune checkpoint inhibitors (ICI), immune-related adverse event(irAE), pneumonitis, programmed cell death 1 inhibitor, cancer immunotherapy

Introduction

Immune-checkpoint inhibitors (ICI) have transformed the treatment of multiple cancer, which significantly improved survival (1–3). One of the remarkable characteristics of ICIs is their relatively mild toxicity profile. Although uncommon, immune-related adverse events (irAEs) may occur in patients receiving immunotherapy, especially in those receiving combined ICI. The mechanisms leading to Checkpoint inhibitor pneumonitis (CIP) are still being clarified. Some potential mechanisms include increasing T-cell activity in response to cross-antigens, increasing levels of autoantibodies and inflammatory cytokines and enhancing complement mediated inflammation (4). Most of irAEs are low grade and can involve almost any organ system. They have uncertain features, but in most cases can be improved by drug immunosuppression and discontinuation of treatment. CIP is uncommon but potentially fatal toxicity of immunotherapy.

The clinical manifestations of CIP are variable, it might show asymptomatic disease, or it may present with respiratory symptoms such as cough, shortness of breath and respiratory failure, in some cases can lead to death. The incidence of CIP in combined therapy (6.5%–10%) was higher than that in monotherapy (3%–4%) (4–6). However, due to the lack of typical imaging findings, the radiological diagnosis of CIP is challenging. In most cases, the main radiologic patterns of CIP include several abnormalities with cryptogenic organizing pneumonia (COP), nonspecific interstitial pneumonitis (NSIP), hypersensitive pneumonitis (HP), ground-glass opacities, and acute interstitial pneumonitis for more severe cases. In some cases, CIP can display a special imaging feature that cannot be classified in any of the above-mentioned specific modes (7). Moreover, CIP is a dynamic process that evolves over time. Most studies reflect the incidence of CIP, but do not clarify the course of it. Here, we report a patient with CIP whose imaging features lag behind clinical symptoms after immunotherapy and radiotherapy.

Case presentations

A 66-year-old man with stage IIIIC lung squamous cell carcinoma (SCC) (cT4N3M0) presented with cough and chest pain for four months (Figure 1). He had a history of smoking for more than 40 years (40 cigarettes a day, quit > 3 months). His body weight was 71 kg, with no significant change from the previous weight. No sensitive gene mutations were detected and the percentage of tumor cells with membranous PD-L1 staining (tumor proportion score) was 30%. Four cycles of tislelizumab plus nab-paclitaxel and carboplatin chemotherapy were performed. The fourth cycle of treatment were synchronized with thoracic radiotherapy (TRT) (60 Gy/30 fractions), the mean lung dose (MLD) was 8.07 Gy, 22.5% of the lung received 20 Gy (V20), and 36% of the lung received 5 Gy (V5), which ended in Jan 2022 (Figure 2C). The patient's symptoms of cough and chest pain improved.

After 20 weeks of the initial tislelizumab administration (20 days after radiotherapy ended), dyspnea and cough developed. The patient presented to our hospital, the laboratory tests including hemogram and inflammatory markers were normal except for the white blood cell of 14.79×10^9/L. Computerized tomography (CT) of the chest showed the tumor was reduced in size and no pneumonitis was observed (Figure 2A). We administered an inhaled acetylcysteine solution for the symptomatic treatment of dyspnea. However, 1 week later, the patient had a fever with a worsening cough and expectation. Due to the Spring Festival, he did not seek medical attention in time. Then his temperature continued to rise. Only 3 days later, in the emergency department, CT was notable for extensive exudation of both lungs, diagnosis of interstitial pneumonia (Figure 2B).

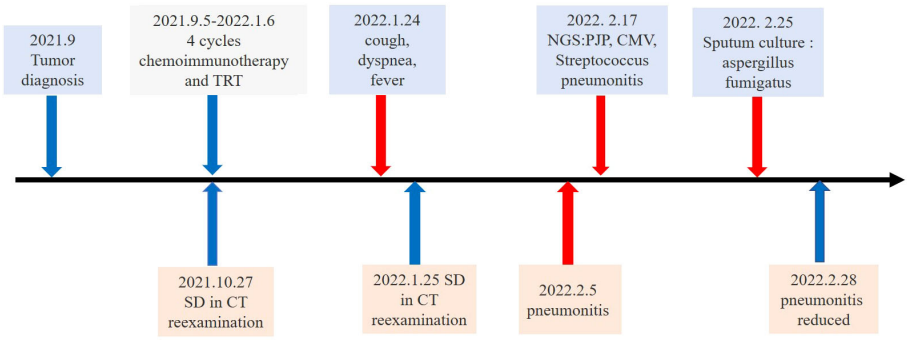

FIGURE 1
Timeline of the major treatment process and CT evaluation of the patient since diagnosis. SD means "stable disease".

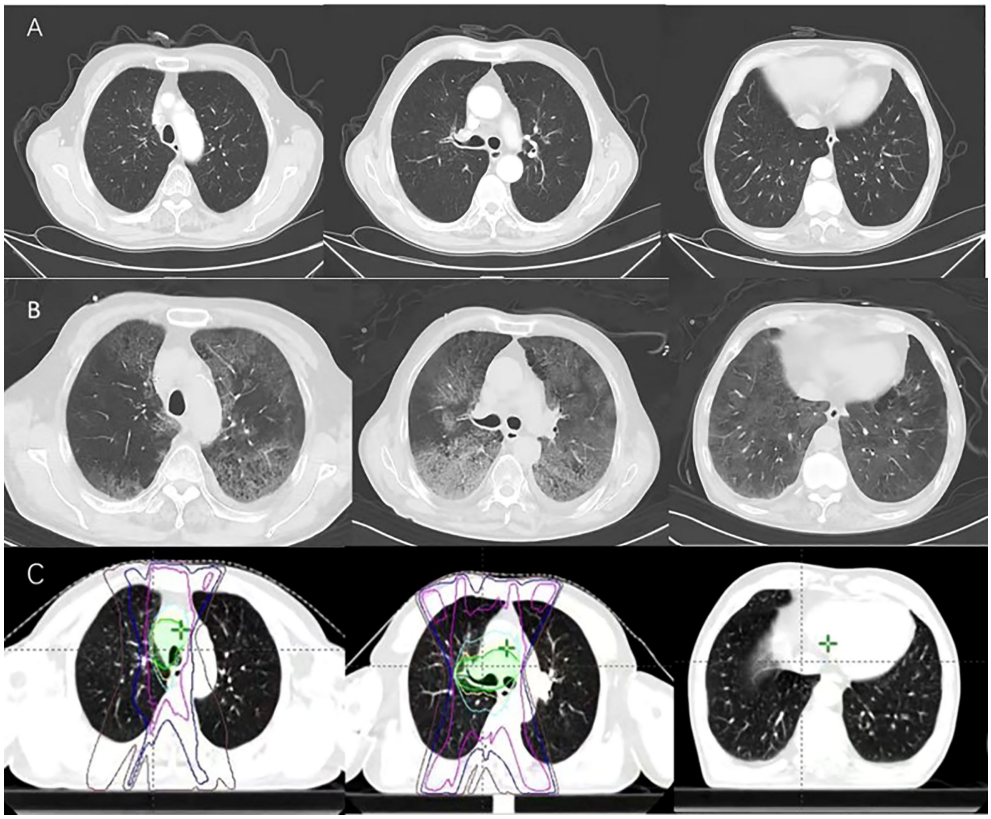

FIGURE 2
(A) 20 days after treatment ends, dyspnea, cough and fever occurred. **(B)** 27 days after treatment ends. **(C)** Radiation field.

The patient's condition continued to deteriorate and he was transferred to the intensive care unit (ICU). His temperature was 42°C, the pulse was 123 beats per minute, mask oxygen was given to the patient. Then his partial pressure of oxygen (PaO2) was only 60 mmHg and the fraction of inspiration O2 (FiO2) was 57%. Serum (1–3)-ß-d-glucan level was 327.12pg/mL, and the galactomannan was 0.25μg/L. According to the multidisciplinary team (MDT), the patient had a history of previous immunotherapy. CT showed large blurred shadow with unclear margins, and his serum (1–3)-ß-d-glucan level are high. It was thought to be pulmonary complications related to immune checkpoint inhibitor therapy and possible coinfection. Then methylprednisolone (40 mg per 12 hours) was administered intravenously. And antibiotics such as Compound Sulfamethoxazole Tablets (Trimethoprim 0.16g and Sulfamethoxazole 0.8g per 6 hours), Sulperazon (3g per 8 hours) and voriconazole (0.2g per 12 hours) were empirically administered. After a week, the patient's condition did not improve, and voriconazole was replaced by caspofungin (70mg once→50mg/day). Bronchoscopy with bronchoalveolar lavage (BAL) was performed. On the 12th hospital day, next-generation sequencing (NGS) analysis of BALF specimens identified Pneumocystis jirovecii(PJP), streptococcus pneumonia and cytomegalovirus (CMV). Ganciclovir (375mg/day for seven days → 0.25g per 12 hours) was added to his treatment and increased the dose of Compound Sulfamethoxazole Tablets (Trimethoprim 0.24g and Sulfamethoxazole 1.2g per 6 hours). At the same time, the patient with sustained grade III/IV thrombocytopenia, immunoglobulin (25 g/day for five days) was injected intravenously. On the 20th hospital day, the fungal and bacterial sputum culture obtained 7 days earlier grew Aspergillus fumigatus. The patient improved after 23 days in ICU and CT showed reduced inflammation.

Discussion

Occurrence of CIP

We described a case of CIP after radiotherapy combined with immunotherapy whose symptoms predate imaging findings, complicated by Pneumocystis, Aspergillus and other infections during the treatment of CIP. Previous studies have shown that during PD-1 inhibitor monotherapy, the incidence

of all-grade pneumonia in NSCLC was 4.1%, and the incidence of grade 3 and above pneumonia was 1.8% (6). ICIs may overstimulate the immune system, change the homeostasis of the host, and lead to excessive inflammatory response. During radiotherapy, ionizing radiation induces free radicals and DNA damage, promoting oxidative stress, vascular damage, and inflammation on normal tissues (8). A pool analysis showed, about 11% of patients who received stereotactic body radiotherapy (SBRT) developed grade ≧2 radioactive pneumonia within a few months (9). The combination of radiation therapy(RT) and ICI works not only by directly tumor-cell killing, but also by stimulating the immune system response through radiation, so as to enhance the ICI effect (10). Many preclinical studies have shown that there is synergistic activity between ICIS and RT. The combination of thoracic radiotherapy and ICI may have synergistic pulmonary toxicity. The occurrence of pneumonitis is an important point worth exploring.

Some studies have demonstrated the safety of radiotherapy combined with immune checkpoint inhibition. In the KEYNOTE-001 trial, the secondary analysis found that prior thoracic radiotherapy was associated with an increased risk of treatment-related pulmonary toxicities (13% vs. 1%, P = 0.046), while there was no significant difference in high-grade pulmonary toxicity (11). The phase 3 PACIFIC trial showed the incidence of any grade pneumonitis/radiation pneumonitis was 33.9% in the immunotherapy group and 24.8% in the placebo group (P value not reported), and the incidence of grade 3 or higher pneumonitis/radiation pneumonitis occurred in 3.4% and 2.6%, respectively (P value not reported) (12). They mainly demonstrated the pulmonary toxicities of sequential combination of ICI with a previous history of radiotherapy. A recent retrospective study in patients who underwent TRT after ICI evaluated the incidence and severity of treatment-related pneumonitis. It showed that the incidence and severity of treatment-related pneumonitis was significantly higher in lung cancer patients who received TRT after ICI (13). As is known, acute "radiation pneumonitis" usually occurs within 4 to 12 weeks after thoracic irradiation. Recently, some studies revealed that pneumonitis in radio-immunotherapy patients usually occurs in previously irradiated areas, regardless of how much time has elapsed after RT, even after a few years. In some patients receiving ICIS, the time interval between radiation therapy and pneumonitis raises the hypothesis of "radiation recall pneumonitis". A recent pooled analysis found that the patients received radiotherapy within 90 days (RT ≤ 90) after ICI had slightly numerically higher rates of pneumonitis than those more than 90 days (RT >90) before the start of ICI therapy. These differences were due to low grade (grade 1-2) AEs (14). In addition, a phase 1 trial of Pembrolizumab combination with chemoradiotherapy for locally advanced NSCLC found pneumonitis of ≥grade 2 occurred in 33% patients, and risk of pneumonitis was higher in patients who started pembrolizumab on day 1 of chemotherapy (15). In summary, the combination of ICI and radiotherapy have a slightly higher rate of pneumonitis than monotherapy, but the difference may be owing to low grade (grade 1-2) AEs pneumonitis that is mostly tolerated. In addition, timing of combination therapy is critical. Longer intervals seem to portend better security. If the interval exceeds 90 days, there is no significant increase in the probability of pneumonitis.

Complex diversity of CIP

The most common symptoms of pneumonitis are dyspnea and cough, fever and chest pain were less common, some patients were asymptomatic at the onset of pneumonitis (16). Several cases of CIP have been reported, indicating that the clinical course of pneumonitis also varies among patients, with some requiring ICU admission, intubation and even death, while others are successfully treated with oral corticosteroid. In addition, a few patients experience recurrent pneumonitis after restarting their immunotherapy (5, 7, 17). Retrospective case studies have inconsistently identified risk factors for underlying lung disease, such as ILD, history of radiation therapy, history of COPD and V20. However, the factors involved are highly variable for individual patients (18).

As far as we know, we present the first case of patient's pneumonitis symptoms prior to radiologic manifestation. In this case, when the patient returned to the hospital with cough, dyspnea, and fever, a CT scan on him found no obvious signs of pneumonitis. However, 1 week later, his symptoms gradually worsened and CT also showed severe pneumonitis. The patient was treated with methylprednisolone (80mg per 12 hours) and empirically administered with antibiotics. His condition had not significantly improved but found coinfection. Regarding infectious complications, it has emerged that the blockade of the PD-1/PD-L1 axis does not appear to inherently increase the risk of infection because it promotes T-cell effector functions. Nevertheless, they can induce irAEs. The treatment of these irAEs require additional immunosuppressive with corticosteroids which can lead to opportunistic infection (19). However, unlike the majority of reported cases, the new reports showed that some infections can be triggered by ICIs without immunosuppressive treatment. It seems that hyperinflammatory dysregulated immunity associated with ICIs drives pathogenesis. These infections are characterized by a dysregulated inflammatory immune response (20).

The patient's NGS analysis also found CMV, there is evidence that CMV may be a potential trigger for severe irAE. A retrospective cohort study comparing CMV infection in patients with different degrees of CIP showed that the CMV positive rate was much higher in patients with severe CIP than in patients with no or mild ICI pneumonitis (91.7 vs. 20%). There are reports that immunotherapy exacerbates progressive fungal

infections, such as aspergillosis, in the absence of immunosuppression. Clinically, this infection can mimic the progression of cancer. Therefore, immune checkpoint inhibition may exaggerate the immune response to fungal colonization, which may promote fungal growth (21).

With rare exceptions, CIP may also be accompanied by other irAE, such as this patient developed thrombocytopenia with normal hemoglobin and normal white cell counts. Bone marrow biopsy showed no obvious morphological abnormalities, no hemophagocytic cells, and no malignant invasion in the patient. Laboratory tests such as antinuclear antibodies were negative, but antiphospholipid and antiplatelet antibodies were abnormal. Thrombocytopenia caused by chemotherapy, infection, or other drugs, was excluded, and the final diagnosis was immune-induced thrombocytopenia. While steroid therapy, he received five platelet transfusions and intravenous immunoglobulin (25 g/day for five days), but the platelets did not recover. In the meantime, the lowest platelet level was 7×10^9/L. Fortunately, his platelets returned to normal after discharge. This also reminds us that in the face of patients after immunotherapy, the adverse reactions may be complex, and we should comprehensively evaluate and designate a comprehensive and individualized treatment plan.

CIP is essentially interstitial pneumonia and opportunistic infections are prone to occur in CIP patients. However, early diagnosis is difficult due to the acute course of infection and the heterogeneity of clinical manifestations. Bronchoscopy and bronchoalveolar lavage (BAL) are key to confirming infection, and NGS should be performed if necessary. We should pay attention to distinguishing between bacterial infection, viral infection, fungal infection and heart failure. Occasionally, patients may present with symptoms without imaging findings, which deserves our vigilance.

Diagnosis and treatment of CIP

The diagnosis of CIP is exclusionary, which is usually a combination of clinical assessment, imaging findings, and laboratory analysis. BAL analysis is also crucial as it can differentiate inflammation and tumor. The BAL cytology shows a predominantly lymphocytic or a mixed pattern (7). Inflammatory markers such as C-reactive protein were also elevated. In some patients who can't have a BAL, imaging findings are crucial to the diagnosis of disease. In imaging, the mainly differential diagnoses of CIP are infection and tumor progression, which can share similar clinical symptoms. The relationship between the radiation field and pneumonia deserves our attention.

To date, the treatment of irAEs has mostly been empirical. Several published guidelines recommended similar treatments for different grades of pneumonitis. Grade 1 pneumonitis is treated by discontinuation of ICI therapy without steroids. Grade 2 pneumonitis can be managed by withholding the ICI therapy and initiating steroids therapy. Grades 3 and 4 pneumonitis should permanent discontinuation of ICI therapy and intravenous steroids along with empirical antibiotic therapy. Therefore, the most common initial treatment for CIP is steroids. But a small proportion of patients are refractory or become resistant to steroids, which is rare but is associated with higher morbidity and mortality (5, 22). For these patients, treatment guidelines suggest second-line immunosuppressive therapy including infliximab, mycophenolate, intravenous immunoglobulin and cyclophosphamide (23–25). Since the clinical course and response pattern to steroids are often individualized. The timing of initiation of additional immunomodulatory agents varies among patients. At present, it is considered that a more effective treatment strategy is to give additional immunomodulatory agents as soon as possible once the patient has the initial signal of refractory response (26). Other promising strategies, such as targeting the microbiome, are emerging and have yet to be included in these guidelines. Multiple case reports of opportunistic infections after ICI treatment by a variety of different pathogens proved the necessity of having a low threshold of investigation for opportunistic infection (20).

As for patients who develop CIP, it has been demonstrated that the use of high-dose glucocorticoids increases the risk of pneumocystis. Thus, PJP prophylaxis has to be considered (27). Increased vigilance and timely identification of pneumonia are prerequisites for early initiation of treatment and prevention of further morbidity and mortality in these patients (28). Because CIP has no clear onset time, patients may have no imaging findings, and even a few patients have symptoms prior to imaging findings. It is recommended that continues to strengthen the monitoring of patient symptoms and signs and laboratory results during immunotherapy (29).

The case has several limitations. The main limitation is due to the serious condition of the patient, methylprednisolone was given before there have a BAL analysis or other microbiology result, which may interfere the BAL cytology. Antibiotics are also used empirically, which prevents us from accurately assessing the initial infection status. In addition, we lack the serial blood gas analysis of the patients, which prevent us to understand the treatment response through PaO2/FiO2. Given these limitations, more CIP patients deserve our attention.

Conclusion

In patients with CIP, a new feature that the clinical symptoms predate imaging findings deserves our attention. CIP is gradually increasing following the use of immune checkpoint inhibitors. When thoracic radiotherapy is combined with ICI, timing of combine is critical, longer intervals seem to portend better security. Clinicians should

strengthen the surveillance and management of pneumonitis. Opportunistic infections are prone to occur in CIP patients and steroid therapy should be used earlier when pulmonary toxicity occurs. It has been demonstrated that the use of high-dose glucocorticoids increases the risk of PJP. Thus, PJP prophylaxis has to be considered. There are critical needs to clarify the mechanisms of CIP and to formulate individualized treatment strategy to improve the safety of tumor immunotherapy. Looking into the future, radiologists must strengthen collaboration with multidisciplinary teams to provide optimal treatment and management for patients with ICI-associated pneumonia, which is so complicated in diagnosis and treatment.

Data availability statement

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

Ethics statement

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

Author contributions

XM proposed, edited, and approved the final manuscript. MY collected clinical information. YW analyzed the data and drafted the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Academic Promotion Program of Shandong First Medical University (2019ZL002), Research Unit of Radiation Oncology, Chinese Academy of Medical Sciences (2019RU071), the foundation of National Natural Science Foundation of China (81627901, 81972863 and 82030082), the foundation of Natural, Science Foundation of Shandong (ZR201911040452). Natural Science Foundation of Shandong (ZR2019MH010), CSCO-Haosen Foundation (Y-HS202102-0089), and CSCO-Xinda Foundation (Y-XD202001-0008).

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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frontiers | Frontiers in Immunology

TYPE Case Report
PUBLISHED 04 January 2023
DOI 10.3389/fimmu.2022.1068978

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Immunology

RECEIVED 13 October 2022
ACCEPTED 05 December 2022
PUBLISHED 04 January 2023

CITATION
Guan S, Zhang L, Zhang J, Song W and Zhong D (2023) A case report of steroid-refractory bullous pemphigoid induced by immune checkpoint inhibitor therapy.
Front. Immunol. 13:1068978.
doi: 10.3389/fimmu.2022.1068978

A case report of steroid-refractory bullous pemphigoid induced by immune checkpoint inhibitor therapy

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The widespread use of immune checkpoint inhibitors in several malignancies has revealed new immune-related adverse events. Bullous pemphigoid (BP) is an antibody-driven autoimmune disease characterized by skin inflammation and fluid-filled bullae. Herein, a 69-year-old man with lung squamous cell carcinoma developed multiple vesicles and tense bullae 3 weeks after the initiation of a programmed death-1 (PD-1) inhibitor, pembrolizumab, and chemotherapy. Biopsy revealed a subepidermal bulla with lymphocytic and eosinophil infiltration, and immunohistochemical studies predominantly showed $CD4^+$ cells, a few $CD8^+$ cells, and the occasional $CD20^+$ lymphocyte. The serum anti-BP180 antibody level, as well as the interleukin-6 and interleukin-10 levels, were elevated compared to the lower levels of tumor necrosis factor-α. Eosinophil levels were high and consistent with the development of blisters. A diagnosis of BP associated with PD-1 inhibitor therapy was made, and the Common Terminology Criteria for Adverse Events classification was grade 3. Immunotherapy was permanently discontinued, and the patient's bullous lesions failed to react to high-dose systemic corticosteroids combined with minocycline and niacinamide. Intermittent blister recurrence occurred in 2 months, eventually improving with the administration of two courses of intravenous immunoglobulin. At 5 weeks of follow-up, the patient's tumor was reduced on a computed tomographic scan. Despite stable BP treatment, however, he repeatedly developed complications due to the complexity of his underlying disease and could not be treated with anti-tumor therapy. Early recognition and management of serious immune-related bullous dermatologic toxicity are essential for patient safety.

KEYWORDS

immune checkpoint inhibitor, bullous pemphigoid, lung carcinoma, pembrolizumab, intravenous immunoglobulin

1 Introduction

Cancer immunotherapy, particularly immune checkpoint inhibitor (ICI) therapy, has revolutionized the treatment of malignancies. These monoclonal antibodies are directed against the inhibitory immune receptors cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) and programmed death-1 (PD-1) and enhance the immune function of T-cells, thus mobilizing the body's immune cells to selectively target cancer cells.

Given their unique mechanism of action, ICIs can also act on a variety of non-pathological human cell types, resulting in immune-related adverse events (irAEs) notably mediated by the triggering of cytotoxic $CD4^+/CD8^+$ T-cell activation. Distinct from the effects of traditional chemotherapy, irAEs tend to have a relatively delayed onset and are inflammatory or autoimmune in nature (1).

Dermatologic toxicities are the most prevalent irAEs associated with ICIs. Incidences of all grades of dermatologic toxicity range from 37%–70% for ipilimumab and 17%–40% for PD-1/programmed death-ligand 1 (PD-L1) inhibitors, respectively. Meanwhile, rates of high-grade dermatologic irAEs range from 1%–3% (2). More than 1/3 of treated patients present mainly with a maculopapular rash and pruritus. The antibody-driven autoimmune disease bullous pemphigoid (BP) induced by ICI therapy is a rare cutaneous irAE. BP is typically less severe than other cutaneous reactions such as toxic epidermal necrolysis (TEN) and stevens-johnson syndrome (SJS) (3). Whereas, for severe or life-threatening BP and all cases of SJS/TEN, hospitalization and permanent ICI therapy discontinuation are required. We present here a severe case of a patient with steroid-refractory BP that began shortly after initiating treatment with the PD-1 inhibitor pembrolizumab.

2 Case presentation

A 69-year-old man with lung squamous cell carcinoma began immunotherapy treatment with pembrolizumab (200 mg) and chemotherapy (albumin-bound paclitaxel) in February 2021 due to 60% PD-L1 expression and a poor Eastern Cooperative Oncology Group (ECOG) performance status of 2. He had received no prior treatment for the cancer. His medical history included diabetes, chronic obstructive pulmonary disease, and chronic eczema of the lower limbs. He was treated with oral hypoglycemic agents (metformin, repaglinide, and acarbose). No regular medication was given for the chronic eczema. He reported no history of autoimmune disorders. He had been a smoker for 1,000 pack-years without quitting.

In the second week of treatment (9 days after the initiation of pembrolizumab and albumin-bound paclitaxel), the patient complained of intense itching initially, then developed extensive erythematous papules and plaques on his trunk and proximal part of the limbs. No improvement was seen with topical corticosteroids. Multiple vesicles and tense bullae, some with adjacent erosions and crusting, developed on his trunk and extremities following discontinuation (~22 days) of pembrolizumab (Figures 1A, B). No oral or ocular mucosal involvement was noted. A biopsy examination of the bulla revealed a subepidermal blister cavity with associated dermal lymphocytic and eosinophil infiltration (Figures 1C, D). In addition, immunohistochemical studies of tissue sections predominantly showed $CD4^+$ cells, a few $CD8^+$ cells, and the occasional $CD20^+$ lymphocytic infiltrate (Figures 1E, F). This revealed that $CD4^+$-mediated immune responses in this patient were significantly enhanced. Laboratory examinations revealed that serum anti-BP180 antibodies (enzyme-linked immunosorbent assay [ELISA]) were elevated to >150 U/mL (normal range, <9 U/mL), while the anti-BP230 antibody (ELISA) concentration was below the detection limit (<5.0 U/mL). Eosinophil levels were high and consistent with the development of blisters. Interleukin-6 and interleukin-10 levels (flow cytometry) were elevated significantly to 37.76 pg/mL (reference range, 0–5.30 pg/mL) and 27.46 pg/mL (reference range, 0–4.91 pg/mL), respectively, compared to the lower level of tumor necrosis factor-α (0.63 pg/mL; reference range, 0–4.60 pg/mL).

As a consequence of these findings, drug-induced BP was diagnosed. Pembrolizumab was discontinued, and the patient's bullae extended after 1 week of oral prednisone 40 mg daily (0.5 mg/kg) and topical high-potency topical steroids (halometasone). Given the patient's severe bullous dermatitis (Common Terminology Criteria for Adverse Events [CTCAE] grade 3, i.e., affecting >30% of the body surface area, limiting self-care activities of daily living), immunotherapy was permanently discontinued, and he was treated with intravenous methylprednisolone 80 mg daily (1 mg/kg) augmented with minocycline and niacinamide.

The patient's erythema became shallow; however, the coverage area was enlarged, and new active bullous lesions appeared. Thus, he underwent another skin biopsy of the edge of the erythema 5 weeks after his last dose of pembrolizumab. Pathology still confirmed BP, with $CD4^+$ lymphocytic infiltrates predominating.

Due to his persistent symptoms, intravenous immunoglobulin (IVIG) was administered at 30 g (400 mg/m^2) once daily for 5 days, resulting in rapid clinical improvement. Control of the BP was subsequently maintained with 40 mg of methylprednisolone daily.

Meanwhile, the patient's respiratory symptoms improved after immunotherapy. At 5 weeks of follow-up, the left lung mass showed partial response (PR) and became an irregularly cave on a computed tomographic scan (Figure 2). However, the patient repeatedly developed complications such as pneumonia, heart failure, blood glucose instability, and type II respiratory failure due to the complexity of his underlying disease and was treated

10.3389/fimmu.2022.1068978

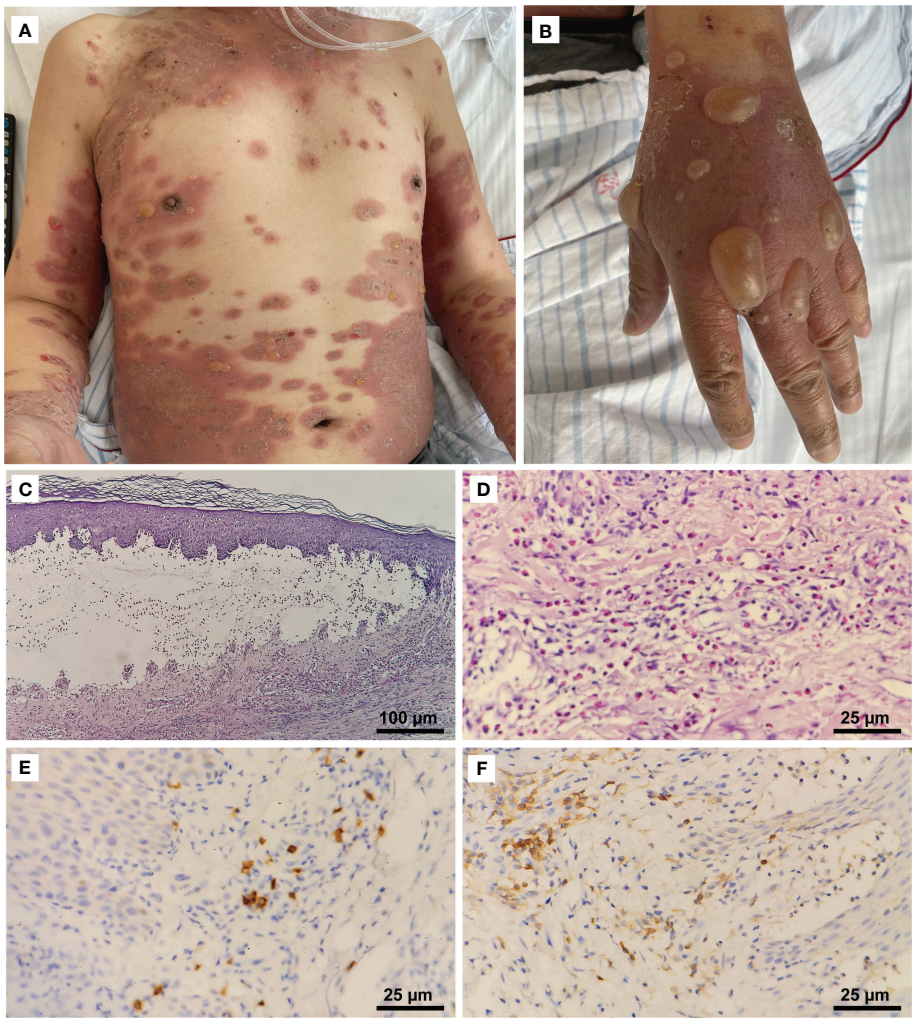

FIGURE 1
Clinical manifestations and histopathological features. **(A)** Erythematous patches and tense bullae on the patient's trunk and extremities (CTCAE grade 3). **(B)** Numerous large tense bullae on the hand. **(C)** Histopathologic examination of a skin lesion on the right hand revealed a subepidermal blister cavity (H&E, 100×). **(D)** Eosinophils infiltrated the upper and mid-dermis (400×). Immunostaining revealed **(E)** a predominantly $CD4^+$ (400×) lymphocytic infiltrate, with **(F)** a few $CD8^+$ cells (400×) and the occasional $CD20^+$ cell.

with prolonged courses of antibiotics; thus, he could not be treated with anti-tumor therapy.

At week 11 (40 days after IVIG administration), the patient developed new vesicles on the anterior thorax and upper limbs again. Eosinophil count in the peripheral blood was concomitantly increased with the activity of the vesicles (Figure 3). He was treated with a second cycle of IVIG, and new blister formation ceased. Unfortunately, the patient died of gastrointestinal bleeding on June 12, 2021 (17 weeks after ICI discontinuation). The case timeline is shown in Figure 4.

A genetic analysis of the patient's blood (via next-generation sequencing) suggested heterozygosity of HLA-A*02:07, HLA-A*11:01, HLA-B*13:01, HLA-B*46:01, HLA-C*01:02, and HLA-C*03:04.

3 Discussion

BP is an autoimmune blistering skin disease characterized by an autoimmune response to the hemidesmosomal protein BP180 within the dermal–epidermal junction that clinically manifests as tense blisters and erosions on the skin. Its typical histological appearance is subepidermal blisters with a dense dermal inflammatory infiltrate mainly consisting of eosinophils and neutrophils (4). Direct immunofluorescence of BP lesions shows deposits of immunoglobulin G and/or C3 along the basement membrane zone. The diagnosis of BP relies on lesion biopsy for hematoxylin and eosin (H&E) staining, perilesional biopsy for direct and indirect immunofluorescence microscopy, and anti-BP180/BP230 ELISA (5). Immune imbalance and antibody

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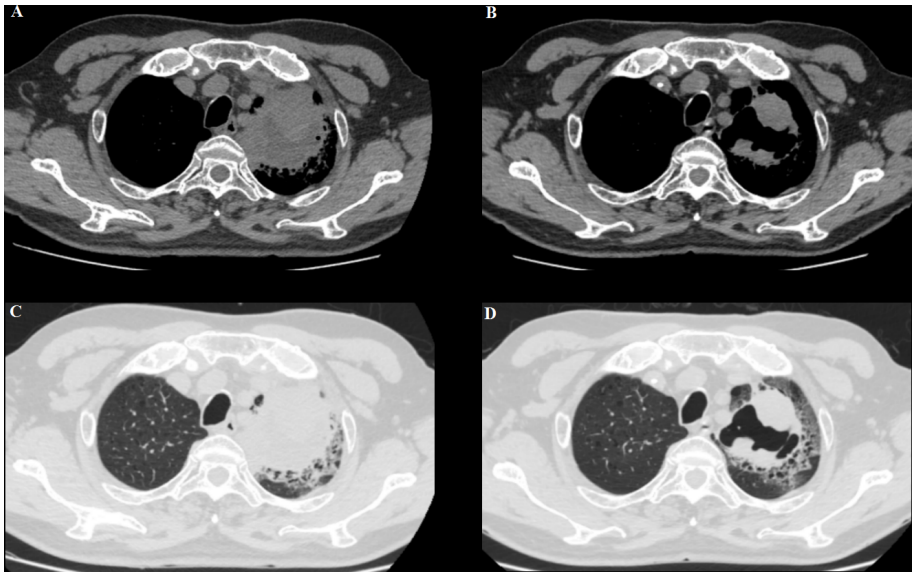

FIGURE 2
(A, C) A baseline computed tomography scan taken before treatment with ICI therapy, demonstrating the left lung mass. **(B, D)** A computed tomography scan taken at the time of BP development (6 weeks after pembrolizumab discontinuation), showing a partial response.

generation are the key pathophysiologies of autoimmune bullous diseases—that is, a breakdown of T- and B-cell tolerance to BP antigens leads to auto-antibody production and subsequent blister induction. Recently, many studies have found that T-cell subsets, which are critical players in autoimmunity, exhibit a range of abnormalities and drive immunopathogenesis and skin inflammation in BP (6). Genetic factors also seem to play an important role in a patient's predisposition to BP. Many polymorphisms of HLA alleles, especially HLA-DQB1*03:01, have been identified in patients with BP across several populations. This allele may thus be involved in the presentation of immunodominant epitopes of BP180 to autoreactive T-cells in BP (7). This association has not been explicitly studied in patients with immunotherapy-induced BP; however, if such an association could be established, extended monitoring for BP may be necessary for some patients being treated with PD-1/PD-L1 inhibitors.

PD-1 or PD-L1 inhibitor use is a known risk factor for drug-induced BP. The latency of bullous disorders due to immunotherapy is generally longer than that of other cutaneous toxicities. In most cases, BP onset is noted concurrently with medication use within 6–8 months of drug initiation (8–10). In this case, however, the bullae first appeared earlier, approximately 3 weeks after the patient's last infusion. Pruritus and other non-specific cutaneous findings may be the only precursors of immunotherapy-induced BP, as was observed here. Therefore, a biopsy is generally required, including both lesional and perilesional sampling of the patient's skin. Given the pharmacodynamics and pharmacokinetics of ICIs, the responses may develop after many weeks or months of immunotherapy, with benefits lasting even after discontinuation. Thus, ICI-induced BP is usually not self-limiting.

ICIs bind to immune checkpoint receptors to reverse the inhibition of T-cell activation, thus restoring anti-tumor immune responses. Removal of this inhibition may also result in both generalized and tissue-specific inflammation, collectively known as irAEs. IrAEs are thought to principally arise from the PD-1/PD-L1 pathway's protection against T-cell–mediated autoimmunity (11). However, B-cells that secrete antibodies may also play a role in the development of irAEs (12). The causal relation between BP and ICIs remains unclear. After ICI usage, epithelial cells and nearby cells may be attacked by the immune system, resulting in

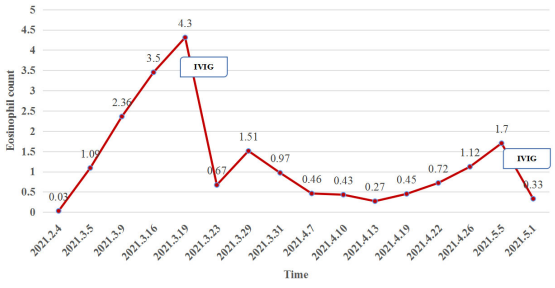

FIGURE 3
The eosinophil count (reference range, 0.02–0.05×10^9/L) in the peripheral blood was concomitantly increased with the activity of the vesicles but plummeted after IVIG administration.

the generation of high levels of CD4 helper T-cell cytokines or increased migration of cytolytic $CD8^+$ T-cells within normal tissues. Previously inhibited T-cells could amplify and improve the activity of the immune system, resulting in the release of auto-antibodies from previously depressed B-cells, thereby triggering irAEs. This reaction may raise a pre-existing auto-antibody response or represent an entirely new process caused by immunotherapy treatment (13). BP180 is an antigen expressed on the surface of malignant melanocytic tumor cells, non–small-cell lung cancer cells, and the basement membrane of the skin. Auto-antibody production against BP180 can weaken the dermal–epidermal junction, leading to a subepidermal cleft of the basement membrane zone.

In our case, though chemotherapy-related cutaneous toxicity cannot be excluded and paraneoplastic BP should be considered, BP induced by anti–PD-1 therapy was favored given the persistent recurrence of bullae over a 2-month period, T lymphocytic infiltrates, and stability of the patient's malignancy with treatment. Immunostaining revealed mostly $CD4^+$ T-cells, with a few $CD8^+$ cells and the occasional $CD20^+$ cell, indicating that a T-cell–mediated immune response predominated in this context. This further confirms the presence of immune-related adverse reactions. Perhaps the refractoriness of our patient's irAE was attributable due to the combined presence of the B-cell–mediated immune response, which may reflect the consequences of unsuppressed cell-mediated immunity, even through the innate immune component of helper T-cells.

Eosinophilic lesional infiltrates and highly peripheral eosinophilia levels are well-known features of BP, and anti-BP180 immunoglobulin E results in essential eosinophil degranulation and consequent blister formation (14). In our case, the change in eosinophil count was consistent with blister development (Figure 3).

Drug-induced BP is characterized by spontaneous exacerbations, typically requiring treatment for 6–12 months involving mainly topical or systemic corticosteroids. Patients with severe bullous dermatitis (G3/4) should permanently discontinue immunotherapy and receive daily treatment with 1–2 mg/kg of prednisone/methylprednisolone. The addition of 1–2 systemic treatment options is often necessary, such as dapsone, azathioprine, mycophenolate mofetil, or doxycyclin. If no improvement is seen after 3 days, suggesting treatment resistance, rituximab and the anti–immunoglobulin E monoclonal antibody omalizumab may be considered (15). IVIG has been used to suppress a wide array of autoimmune and chronic inflammatory conditions. The immunomodulatory mechanisms of IVIG include modulating the activity and effector functions of B and T lymphocytes, impacting antigen presentation, pathogenic auto-antibodies, the complement system, and cytokines (16).

In most reported cases, BP during ICI therapy has been relatively mild. Systemic and topical corticosteroids were the most common treatments, whereas biologic and targeted agents were used predominantly in cases refractory to treatment with corticosteroids (10). Our patient exhibited severe and extensive bullous lesions refractory to high-dose corticosteroids (1 mg/kg). Despite combining therapy with minocycline and niacinamide, the patient's BP still did not respond well. To clarify the immune microenvironment of BP, we performed immunostaining, which confirmed that the infiltrating lymphocytes were mainly $CD4^+$ T-cells, with the occasional $CD20^+$ T-cell also identified, further guiding the treatment of BP. IVIG was administered instead of the anti-CD20 monoclonal antibody rituximab. With the application of two cycles of IVIG, the bullae were finally controlled, and the hormone dose (40 mg of methylprednisolone daily) was gradually tapered off. Considering the tumor response, when BP was present, the patient's tumor mass shrank significantly. A retrospective study suggested that patients who develop ICI-induced BP may experience improved tumor responses and survival outcomes compared to those who do not (17). In addition, retrospective data generally suggest that immunosuppressive

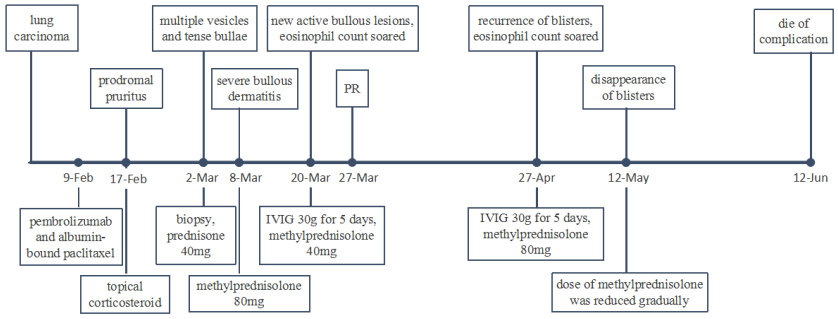

FIGURE 4
Case timeline. PR, partial response.

therapy (primarily systemic corticosteroids) initiated after irAE onset does not appear to decrease ICI efficacy (18).

What clinicians are most concerned about is whether ICI therapy can be continued. The mean elimination half-life of both pembrolizumab and nivolumab is approximately 26 days, but the effects of PD-1 inhibitors almost certainly last longer. The persistence of lesions several months after ICI therapy discontinuation has also been reported (17). Significant irAEs often necessitate significant caution regarding the rechallenge of immunotherapy.

There are a few limitations to our study. Given its rarity, no disease-specific guidelines exist for the treatment of ICI-induced BP, and it remains uncertain whether immunohistochemical staining of lesion pathology could guide biological and targeted treatment of such. The current knowledge base of all aspects of ICI-induced BP is limited. Clinicians should be aware of the early recognition and grading of irAEs, timely treatment, and individualized management with immunosuppression and ICI administration. Even following ICI therapy discontinuation, aggressive management to prevent extensive disease with high morbidity is pivotal to optimize clinical efficacy and safety.

Data availability statement

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

Ethics statement

The studies were reviewed and approved by The Ethical Committee of Tianjin Medical University General Hospital (Ethical NO. IRB2022-WZ-208).The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

SG, LZ and DZ designed and wrote the initial draft of the manuscript. Images of histology slides were created and captioned by WS. SG, LZ, JZ and DZ revised the paper. DZ leads a multidisciplinary team to guide patient management of irAEs for oncology patients being treated with immune checkpoint inhibitors at Tianjin Medical University General Hospital. All authors contributed to the article and approved the submitted version.

Conflict of interest

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

Publisher's note

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frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 10 January 2023
DOI 10.3389/fimmu.2022.1072612

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Immunology

RECEIVED 17 October 2022
ACCEPTED 19 December 2022
PUBLISHED 10 January 2023

CITATION
Pan L, Meng F, Wang W, Wang X-h, Shen H, Bao P, Kang J and Kong D (2023) Nintedanib in an elderly non-small-cell lung cancer patient with severe steroid-refractory checkpoint inhibitor-related pneumonitis: A case report and literature review. *Front. Immunol.* 13:1072612. doi: 10.3389/fimmu.2022.1072612

Nintedanib in an elderly non-small-cell lung cancer patient with severe steroid-refractory checkpoint inhibitor-related pneumonitis: A case report and literature review

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Immune checkpoint inhibitors tremendously improve cancer prognosis; however, severe-grade immune-related adverse events may cause premature death. Current recommendations for checkpoint inhibitor-related pneumonitis (CIP) treatment are mainly about immunosuppressive therapy, and anti-fibrotic agents are also needed, especially for patients with poor response to corticosteroids and a longer pneumonitis course. This is because fibrotic changes play an important role in the pathological evolution of CIP. Here, we report a case demonstrating that nintedanib is a promising candidate drug for CIP management or prevention, as it has potent anti-fibrotic efficacy and a safety profile. Moreover, nintedanib could partially inhibit tumor growth in patients with non-small-cell lung cancer, and its efficacy can be improved in combination with other anti-tumor therapies.

KEYWORDS

checkpoint inhibitor-related pneumonitis, nintedanib, pulmonary fibrosis, non-small-cell lung cancer, immunotherapy

Highlights:

· Steroid-refractory CIP in patients with non-small-cell lung cancer (NSCLC) may be particularly lethal owing to progressive respiratory failure and compromised immune system during immunosuppressive treatment of CIP.

· Nintedanib showed great efficacy with good tolerance in treating sustained interstitial fibrosis and reducing pulmonary function decline in the long course of CIP.

· Nintedanib is an anti-tumor-supporting agent in patients with NSCLC, which can positively control tumor growth in combination with chemotherapy and might boost immunotherapy and prevent CIP when used with immune checkpoint inhibitors.

1 Introduction

Immune checkpoint inhibitors (ICIs), including programmed cell death 1 (PD-1) or its ligand programmed cell death ligand 1 (PD-L1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4) inhibitors, have made major breakthroughs in improving the progression-free survival (PFS) and overall survival (OS) rates of lung cancer patients (1). The US Food and Drug Administration has moved quickly to incorporate ICIs as a first-line treatment for advanced non-small-cell lung cancer (NSCLC) (2). However, toxicities from ICIs that lead to immune-related adverse events (irAEs) and even fatal adverse events (FAEs) pose a great challenge to immunotherapy (3, 4). Approximately 46.2% of FAEs involves the respiratory system (5, 6). Checkpoint inhibitor-related pneumonitis (CIP) ranges in severity from mild and self-limiting (grade 1–2) to fulminant and life threatening (grade 3–4) and often necessitates immunomodulatory treatments (5, 7, 8). The incidence of any grade CIP in patients with NSCLC ranges from 2% to 39.3% and that for grade ≥3 CIP is approximately 0.6%–4% (9, 10). The mean time from ICI initiation to CIP onset is approximately 10 weeks (2.5 months) (9). The overall fatality rate of any CIP grade ranges from 10% to 27% (5, 10, 11).

Patients with NSCLC are at risk of a higher incidence and severity of CIP (11) owing to increased susceptibility to frequent tobacco exposure (12) and/or underlying chronic respiratory diseases [chronic obstructive pulmonary disease (13), pulmonary fibrosis (14) and tumoral involvement (15)]. Previous real-world studies revealed that patients with NSCLC with CIP had a higher overall response rate but a significantly shorter overall survival after ICI initiation than those without CIP (16–18). Thus, this highlights the importance of better management of CIP to achieve the best outcome of immunotherapy.

Corticosteroids are the core treatment for any grade of CIP according to the current guidelines (19–21). Some patients might be steroid refractory (22) and require other immunosuppressive agents to control the rapid progressive symptoms (especially for those in severe grades) (20), leading to a higher mortality during CIP treatment (12). Some researchers have proposed classifying the clinical phenotype of CIP as acute, subacute, and chronic phases and have divided the pathological process of CIP into inflammatory, profibrotic, and fibrotic stages (23, 24). These concepts provide a new direction for CIP treatment besides immunosuppression therapy, which is anti-fibrotic treatment.

Nintedanib is an oral triple angiokinase inhibitor (25) and has been approved for the anti-fibrotic treatment of patients with idiopathic pulmonary fibrosis (IPF) and chronic interstitial lung diseases (ILDs) (26), even for those with a progressive phenotype (27). Nintedanib is also currently used in patients with coronavirus disease 2019 (COVID-19) to significantly reduce the duration of ventilator use and improve imaging performance (28). In addition, nintedanib has shown sufficient antitumor efficacy in patients with NSCLC (29) and has been approved in the European Union for the treatment of patients with lung adenocarcinoma following first-line chemotherapy (30). Thus, we hypothesized that nintedanib has dual favorable anti-fibrotic and anti-tumor efficacy in patients with NSCLC-CIP and shows good tolerance.

Herein, we report a case of severe steroid-refractory CIP in an elderly patient with NSCLC treated with a PD-1 inhibitor. The patient's condition was successfully improved by immunosuppression therapy (high-dose corticosteroids and infliximab), medical intensive care unit (MICU) support, and nintedanib administration. We also present a literature review of the contradictory clinical outcomes of CIP in patients with NSCLC and the potential pathogenic mechanisms of CIP. Our findings suggest that nintedanib is a potential anti-fibrotic agent that could act as an important supportive drug in alleviating chronic fibrotic development of CIP and could exert certain antitumor efficacy under critical conditions of severe CIP with good tolerance.

2 Case presentation

2.1 The basic condition and diagnosis

The patient was a 72-year-old male heavy smoker (50 pack-years). During a routine examination in December 2020, a space-occupying lesion (2.9 cm × 2.6 cm) near the lung hilum (Figures 1A, F) and mild fibrotic changes of unclear origin were detected (Figures 2A, F). He underwent EBUS-TBNA pathological examination and PET-CT to confirm the final diagnosis of squamous cell lung carcinoma (SqCLC; T1N1MO, stage IIb). Genomic sequencing test showed no driver mutation for targeted therapy (*ALK(−), BRAF(−), BRCA1(−), BRCA2(−), EGFR(−), ERBB2(HER2)(−), FGFR2(−), FGFR3(−), KIT(−), KRAS(−), MET(−), NRAS(−), NTRK1(−), NTRK2 (−), NTRK3 (−), PDGFRA(−), RET(−), ROS1(−)*, and *IDH2(+)*); however, the patient tested positive for PD-L1 (TPS=25%, IPS<1%, tested using a Ventana SP263 assay), with tumor mutation burden of 7.26 Muts/Mb (tested using a next-generation sequencing (NGS) panel and paired with peripheral blood sample sequencing). Other genetic mutations related to immunotherapy were also

tested as follows: *CD274(−), PDCD1LG2(−), MLH1(−), MSH2(−), MSH6(−), PMS2(−), POLD1(−), POLE(−), TP53(+), ATM(−), ATR(−), BRIP1(−), CHEK2(−), FANCA(−), RAD50(−), PALB2(−), CHEK1(−), MRE11(−), PBRM1(−), MDM2(−), MDM4(−), DNMT3A(−), JAK1(−), JAK2(+), PTEN(−), STK11(−), CCND1(−), FGF19(−), FGF3(−), FGF4(−)*. The patient had no history of cardiopulmonary or connective tissue disease, and the Eastern Cooperative Oncology Group (ECOG) performance status was rated as 2 points.

2.2 Two cycles of PD-1 inhibitor + nab-paclitaxel → progressive respiratory failure

Owing to the patient's age and tumor location, systemic chemotherapy combined with immunotherapy was administered: intravenous infusion of albumin-paclitaxel 160 mg (D1, D8) combined with camrelizumab 200 mg (D1). After two cycles of the above treatment, the patient developed fever after fatigue and colds on 13 February, 2021, with a maximum temperature of 39°C and shortness of breath. The patient was admitted immediately the next day. Bilateral bronchial breathing sounds and Velcro rales were detected. Laboratory examination revealed an inflammatory blood reaction (Figures 3A, B), decreased oxygen partial pressure (Figure 3C), decreased oxygen saturation (85% SaO_2), and increased D-dimer levels (≥20 μg/ml) (Figure 3D). Images showed that the primary cancer foci shrank significantly and were surrounded by patchy ground-glass opacities (GGOs) and consolidation shadows (Figures 1B, G). Bilateral peribronchovascular and subpleural GGOs, reticulation, and consolidation were predominant in the middle to lower lungs (Figures 2B, G). Contrast-filling defects were also observed in the pulmonary artery branches of the upper and lower lobes of the left lung. The patient was preliminarily diagnosed with type I respiratory failure and interstitial pneumonia (CIP?), pulmonary embolism, and SqCLC (T1N1MO stage IIb, partial response [PR]). The patient was administered with mask oxygen inhalation therapy (8 L/min), systemic corticosteroid pulse therapy (methylprednisolone sodium succinate, 240 mg Q.D. for 2 days), and anticoagulation treatment (enoxaparin sodium, 4,000 U Q.D.) on February 16. Considering that infection could not be ruled out, empirical anti-inflammatory and antiviral treatments were administered, along with anti-asthmatic, gastric-protecting, calcium-supplementing, and other symptomatic support treatments. After 48 h, respiratory failure progressively aggravated, and the oxygenation index ($PaO2/FiO_2$) was calculated as 125 mmHg. The patient was then transferred to the MICU for further treatment on February 18.

2.3 In MICU: Steroid-refractory CIP grade 4 + compromised immune system

The patient was intubated for mechanical ventilation. Bedside chest radiography revealed poor lung transparency (Figure 4A). As a diagnosis of exclusion, we considered that the pulmonary symptoms and signs were caused by ICI toxicity and responded inadequately to high-dose corticosteroids. The patient had no signs of other concurrent irAEs, such as dermatitis, hepatitis, nephritis, or myopericarditis. The final diagnosis was steroid-refractory CIP grade 4 (G4), defined as a life-threatening respiratory compromise. For steroid-refractory CIP G4, infliximab (500 mg for 1 day) was administered intravenously, and the ICIs were permanently discontinued. Oral corticosteroids were slowly tempered. Two days after

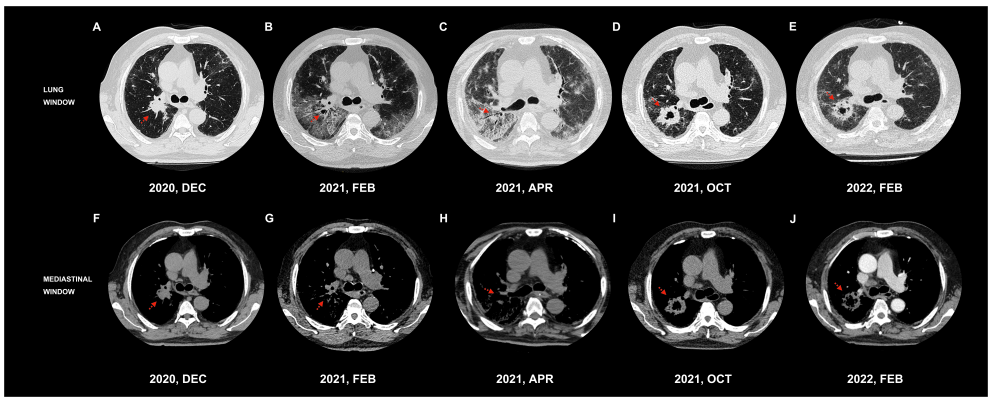

FIGURE 1
Chest CT of tracheal carina level in the lung and mediastinum windows. dynamic changes of the space-occupying lesions. **(A)**/**(F)** A space-occupying lesion (2.9 × 2.6 cm) in the upper lobe of the right lung. **(B)**/**(G)** The primary foci shrank significantly and was surrounded by GGOs and consolidation shadow. **(C)**/**(H)** Multiple GGOs, reticulation, grid lesions, and traction bronchiectasis in the dominant lobe around the primary foci with patchy lesions in the opposite lobe. **(D)**/**(I)** A recurred lobulated mass (5.0×2.6 cm) in the upper lobe of the right lung. **(E)**/**(J)** Mildly reduced lesion (4.2×4.3 cm).

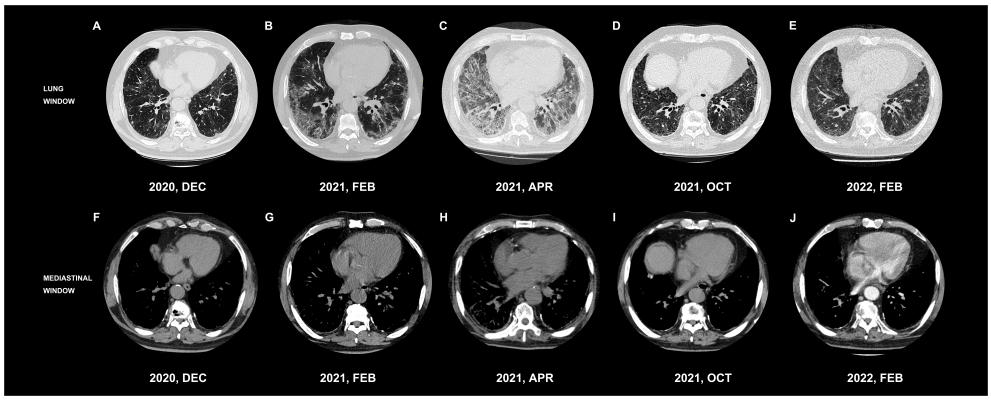

FIGURE 2
Chest CT of inferior lungs in the lung and mediastinum windows. Dynamic changes in the interstitial lesions in the bilateral inferior lobes of the lung. **(A)**/**(F)** Mild basic interstitial changes. **(B)**/**(G)** Significant peribronchovascular and subpleural GGOs, reticulation, and consolidation. **(C)**/**(H)** Significant fibrotic with GGOs, reticulation, consolidation, honeycomb shadows, and thickened interlobular septa. **(D)**/**(I)** Significantly improved interstitial changes and better transparency. **(E)**/**(J)** Stable mild interstitial changes.

immunomodulatory treatment, the inflammation indicators increased again (Figures 3A, B), and plenty of sticky sputum was collected from the patient. We performed sputum culture and sputum smear and found *G+ cocci*, *G− bacillus*, *Acinetobacter baumannii*, and *Candida albicans*. We then examined the bronchoalveolar lavage fluid (BALF) using NGS and found *Acinetobacter baumannii*, *C. albicans*, and *Enterococcusum faecium* infection (Table 1).

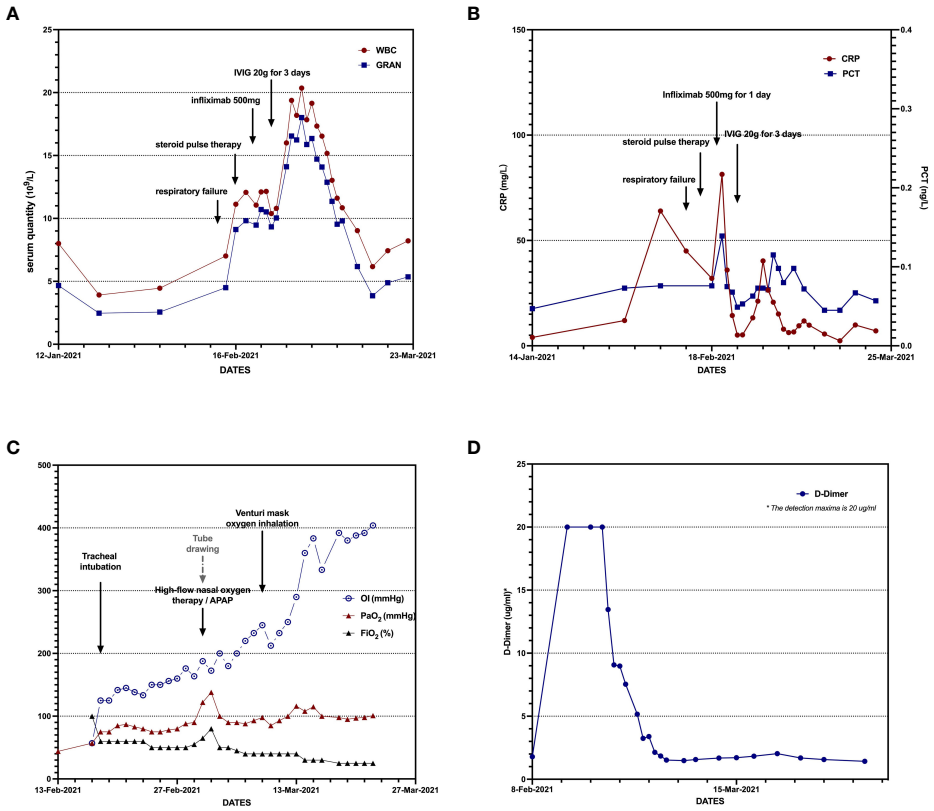

FIGURE 3
Dynamic changes in laboratory examination: inflammatory reaction, hypoxia with hypercoagulable state **(A–D)**.

Considering severe comorbid infectious pneumonia due to compromised immune system, the patient was administered with tigecycline combined with meropenem as anti-inflammatory therapy, micafungin as antifungal therapy, and intravenous immunoglobulin pulse therapy for 3 days to enhance the self-immunological barrier. Enoxaparin sodium anticoagulant therapy was continued for pulmonary embolism. After 3 days, the temperature and laboratory indicators improved (Figure 3). Bedside chest radiography showed improved bilateral lung transmittance (Figure 4B). On March 2, endotracheal intubation was removed, and the patient was administered with nasal high-flow oxygen (45 L/min, oxygen concentration 50%).

Figure 5 shows the management axis of CIP G4 and the critical complications in this patient. After sufficient medical care in the MICU, the patient's condition stabilized, and he was transferred to the general ward on March 9. To manage pulmonary interstitial fibrotic changes and potentially inhibit tumor growth, nintedanib 150 mg was prescribed orally twice daily during the subacute phase of CIP. With good tolerance, the dosage was increased to 200 mg orally twice daily 1 week later and has been maintained to date.

2.4 SqCLC maintenance therapy: nab-paclitaxel + nintedanib + afatinib

On 2 April 2021, the patient's ECOG score was rated 4 before discharge; lung CT reexamination showed no new space-occupying lesions, and the original occupation was significantly smaller than the previous one in December 2020 (Figures 1C, H). The therapeutic efficacy of SqCLC was evaluated as PR. For CIP, pulmonary injury had evolved into an organizing and fibrotic stage. Multiple GGOs, reticulation, grid lesions, and traction bronchiectasis in a cryptogenic organizing pneumonia (OP) pattern were found in the dominant lobe around the primary tumor site, with patchy lesions in the opposite lobe (Figures 1C, H). The bilateral lower lungs were predominantly fibrotic with GGOs, reticulation, consolidation, honeycomb shadows, and thickened interlobular septa (Figures 2C, H).

Owing to the patient's deteriorating physical status and COVID-19 public control policy, the patient had recuperated at home for 6 months and maintained oral nintedanib therapy (200 mg twice daily), with slow tapering of oral corticosteroid therapy. The patient visited the hospital for a review on 13 October 2021. The performance status improved, with an ECOG score of 2. The CT scan showed a lobulated mass with a size of 5.0 cm × 2.6 cm in the upper lobe of the right lung, surrounded by fine burrs and thick-walled cavities (Figures 1D, I). The interstitial changes in the bilateral lower lobes were significantly improved compared to the previous films (Figures 2D, I). There were swollen lymph nodes in the mediastinum, and the largest one was approximately 2.6 cm × 2.0 cm in size. Moreover, the levels of serum tumor markers increased (U/L): CEA, 16.6; AFP, 2.55; CA125, 102; CA153, 36.1; CYFRA, 9.43; NSE, 20.00; and SCCA, 13.6.

The therapeutic efficacy of lung cancer was evaluated as progressive disease (PD), with T2bN2M0 stage IIIa. After consultation with the patient's family, the third chemotherapeutic cycle, comprising albumin–paclitaxel, was started at 200 mg (D1, D8/q3w), and oral nintedanib treatment was continued. In addition, afatinib 30 mg orally once daily was administered as second-line cancer therapy. The patient received four to seven cycles of albumin-paclitaxel treatment on 11 and 30 November 2021, 22 December 2021, and 21 February 2022.

Lung CT examination showed that the former shadow was 4.2 cm × 4.3 cm in size (Figures 1E, J) and stable mild interstitial changes (Figures 2E, J). The largest lymph node in the mediastinum was approximately 2.4 cm × 1.8 cm in size. The remaining abdominal CT, brain MRI, and whole-body bone ECT scans showed no obvious abnormalities or metastasis. A response of stable disease was achieved. The patient showed mild adverse reactions: slightly increased levels of liver transaminase (72 U/L AST (15–40) and 69 U/L GGT (10–60). To date, the

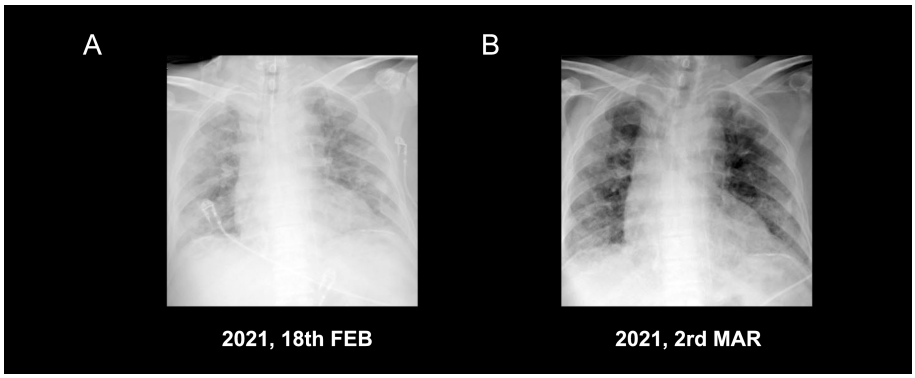

FIGURE 4
Bedside chest radiograph: both lung translucency and consolidation shadow decreased after treatment **(A, B)**.

TABLE 1 Detected bacteria and fungi in BALF by NGS.

Type[a]	Species		Genus	
	Name	Number of detected sequences[b]	Name	Number of detected sequences[b]
G^-	*Acinetobacter*	221,142	*Acinetobacter baumannii* *Acinetobacter nosocoomialis*	116,450 2,200
G^+	*Enterococcus*	86	*Enterocooccusum faecium*	33
G^-	*Dialister*	39	*Dialister pneumosintes* *Dialister invisus*	33 6
G^-	*Streptobacillus*	27	*Streptobacillus notomytis*	27
G^-	*Anaeroglobus*	13	*Anaeroglobus geminatus*	13
Fungi	Candida	139	Candida albicans	128

Type [a]: G^+ → Gram-positive bacteria/G^- → Gram-negative bacteria.
number of detected sequences [b]: refers to the number of strictly matched sequences of the microorganism detected at the genus/species level.

patient has maintained the current lung cancer treatment regimen. The process of antitumor therapy for the patient is shown in Figure 6.

3 Discussion

By inhibiting PD-(L)-1 and CTLA-4, ICIs can facilitate immune surveillance and enhance immune attack in the tumor immune microenvironment (12). However, ICI-activated immune mechanisms may attack normal tissues by identifying cross-antigens between normal and tumor cells (31), leading to various types of irAEs (32). The occurrence of irAEs may indicate that immunotherapy has already activated the immune system of patients and is attributed to better tumor clearance effects. A meta-analysis including 4,971 cancer patients found a significant association between irAE occurrence and reduced risk of tumor progression after receiving ICIs (16). Multiple retrospective analyses also reported that the development of irAEs was associated with better survival outcomes in patients with NSCLC treated with PD-(L)-1 inhibitors (17, 33), and patients with greater toxicity to ICIs could attain better tumor-killing effects (18). CIP might occur more often and have a faster onset in NSCLC than in other types of cancer (32). Moreover, CIP is especially associated with better ICI efficacy than any other type of irAE in patients with NSCLC (34). In addition, the occurrence of CIP might suggest that patients with NSCLC can achieve a prolonged tumor-regression duration after ICI discontinuation (18, 35, 36).

However, among the cases of CIP with a variety of malignancies, CIP-related death was mainly observed in patients with NSCLC (15, 37). Two meta-analyses including more than 8,000 patients found that the occurrence of low-grade irAEs/CIP (grade 1–2) was significantly associated with a

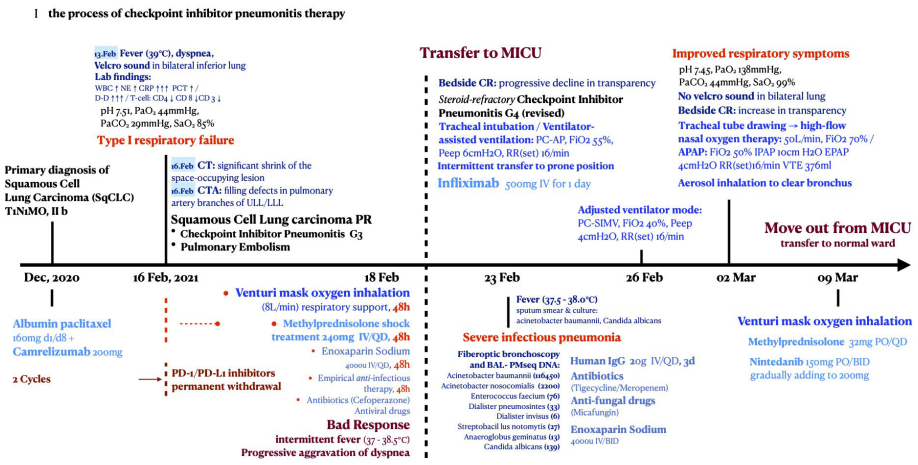

FIGURE 5
The management axis of acute severe steroid-refractory CIP.

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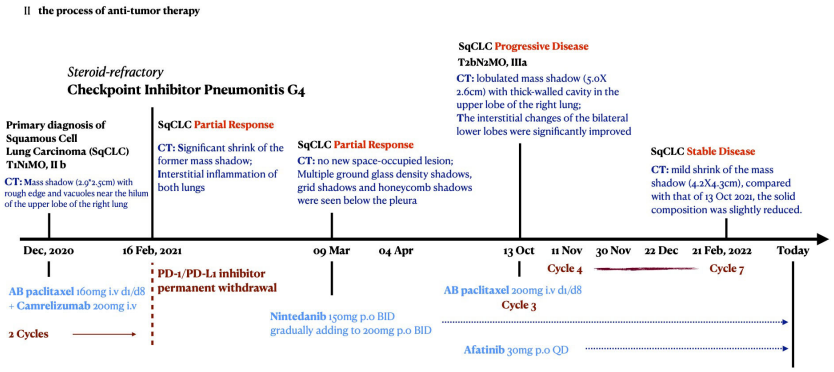

FIGURE 6
The time axis of anti-tumor therapy.

favorable OS and PFS, whereas severe-grade irAEs/CIP (grade 3–4) was not significantly associated with OS but was significantly associated with a favorable PFS and better overall response rate (16, 33, 36). Low-grade CIP is usually mild and easily manageable, with a shorter pneumonitis course and less impairment of the pulmonary function (11). Severe-grade (with or without steroid-refractory) CIP greatly increases the mortality risk of patients with NSCLC during ICIs treatment owing to overlapping risk factors on the respiratory system (9, 38), compromised pulmonary function (15, 39), and progressive respiratory failure (24), despite the good inhibitory effects of ICIs on the primary tumor.

This contradiction unveils the importance of a better clinical strategy for CIP management (especially severe-grade and/or steroid-refractory CIP) to obtain the best clinical efficacy from immunotherapy in patients with NSCLC. Therefore, a deeper understanding of the onset and progression of CIP and its various clinical manifestations is necessary to identify new treatment strategies. Here, we simply analyzed the pathological mechanisms in different stages of CIP disease progression and the histological and imaging manifestations of each stage. We focused on the mechanisms of the (pro)fibrotic stage in the pathological process of CIP and its possible interacting pathways with the tumor in the lung microenvironment.

Current guidelines for CIP management mainly focus on immunomodulatory therapy to relieve the onset of symptoms. In this case, we used high-dose corticosteroids and infliximab (a chimeric immunoglobulin G (IgG) monoclonal antibody directed at tumor necrosis factor alpha (TNF-α)) to control the rapid progressive respiratory failure of the patient and supported him immediately with critical care in the MICU. He was found to be infected with *Acinetobacter baumannii*, *Candida albicans*, and *Enterococcusum faecium* in the lung, probably due to a compromised immune system after sufficient immunosuppression and was in a very critical condition. As experienced respiratory physicians, we supported him with flexible mechanical ventilation and prone position ventilation, with comprehensive systemic anti-infective therapy, immunomodulatory therapy, and life-support treatment. This patient improved after 1 week and showed a prominent organizing pneumonia pattern in images with GGOs, consolidation, reticulation, thickened stripes, and grids. However, there is no authoritative definition or treatment recommendation for this chronic fibrotic condition of CIP, especially for steroid-refractory patients, for whom slowly tapering oral corticosteroids cannot play a leading role in further treatment.

Based on our knowledge of the treatment of lung fibrotic disorders and lung cancers, we chose nintedanib as an important supportive drug to manage the fibrotic changes and we hoped that it could potentially inhibit tumor recurrence during recuperation. Herein, we also illustrated previous positive clinical and experimental evidence to support nintedanib as a new therapeutic consideration in CIP management, which has been approved as one of the only anti-fibrotic drugs for progressive interstitial lung diseases (ILDs) (27, 40) and idiopathic pulmonary fibrosis (IPF) (41, 42) and has been recognized as a promising anti-tumor drug for advanced patients with NSCLC (mainly adenocarcinoma) in combination with docetaxel (43, 44). Nintedanib also showed interesting performance in current studies, as it significantly enhanced immune recognition of pulmonary cancerous cells (45, 46) and significantly reduced lung consolidation when combined with immunotherapy (45).

Studies have reported that the incidence of CIP is more often associated with smoking history (47), age over 70 years (38), squamous cell carcinoma subtype(> adenocarcinoma subtype) (31, 48, 49), combination therapy (> ICI monotherapy) (50, 51), PD-1 inhibitors (> PD-L1 and CTLA-4 inhibitors) (51, 52), the presence of baseline fibrosis on chest CT scans (11, 53), and pre-existing pulmonary diseases including IPF, ILDs, chronic obstructive pulmonary

disease (COPD), and asthma (14, 13, 54). As we could see, the patient in this case had been exposed to a high risk of CIP and then developed severe steroid-refractory CIP, while we could not provide any preventive treatment before CIP onset, as there were no such recommendations in the previous studies. Nintedanib might be a strong candidate to prevent CIP by applying it in combination with ICIs in patients with NSCLC with a high risk of developing CIP.

3.1 Pulmonary injury evolution stages of CIP

The most common symptoms of CIP are non-specific, such as cough, expectoration, and shortness of breath (9, 55). Radiographic features that indirectly reflect the most prominent pulmonary histopathological changes are important for the diagnosis and grading of severity in clinical settings. Despite the huge heterogeneity, some scholars have recognized the following imaging patterns in patients with CIP: acute interstitial pneumonia (AIP), acute respiratory distress syndrome (ARDS), diffuse alveolar damage (DAD), non-fibrotic or fibrotic hypersensitivity pneumonitis (HP), cryptogenic organizing pneumonia (OP), non-specific interstitial pneumonia (NSIP), and other unclassified types (31, 55–57). These patterns can be classified into different evolution phases or different toxicity grades, from the excessive inflammatory stage or the highest grade (AIP/ARDS/DAD) to the proliferative organizing (nonfibrotic HP/OP) and fibrotic stages (fibrotic HP/NSIP) in lower grades (31, 57, 58). In general, all histopathological changes develop from abnormal inflammation resulting from an ICI-activated immune system. Similar to wound healing in lung injuries, mild or severe pulmonary fibrotic changes would always accompany the exaggeration or regression of inflammation (59, 60), which means that inflammatory, organizing, and fibrotic changes could occur simultaneously within the lung tissue (61, 62). The severity and duration of inflammation and the rate of absorption of the damaging exudation determine the clinical and pathological features of CIP in the initial episode before treatment.

The current treatment strategy for CIP mainly involves negative immunomodulatory therapy based on the symptom grade from G1 to G4, including pulse and/or tapering corticosteroids, ICI discontinuation, and immunosuppressant addition (19, 20). Immunosuppressants are usually temporarily applied in steroid-refractory patients who present inadequate response after 48–72 h of corticosteroid treatment (21). Immunosuppressants should be cautiously used, considering their potent immunosuppressive efficacy and unclear negative effects on patients with NSCLC-CIP. First, excessive suppression of the immune system could lead to opportunistic infections very quickly (63–65), which would prolong the duration of critical conditions and increase the complexity of treatment. Second, durable antitumor activity might exist after discontinuation of ICI therapy (35, 66, 67), whereas immunosuppressants, such as infliximab, could weaken the ongoing antitumor immune activity initially launched by ICI treatment (22). As mentioned previously, patients with a severe-grade CIP might achieve better tumor remission, although the clinical symptoms of CIP are life threatening. However, this advantage can be counteracted by CIP treatment, leading to an extremely poor prognosis. These findings are consistent with clinical data. Previous real-world studies have reported that more than half of patients with grade 3 CIP died during ongoing corticosteroid treatment (11) or after receiving additional immunosuppressive drugs (4). In addition, infliximab can cause interstitial lung disease (ILD) (68), which increases the risk of interstitial fibrosis.

In this case, the patient developed secondary *A. baumannii*-induced pneumonia beyond severe-grade CIP and experienced severe interstitial fibrotic changes after receiving high-dose corticosteroids and infliximab. There are no clear recommendations for the sequential maintenance strategy of steroid-refractory CIP patients, especially for those with severe grade. Neither the optimal corticosteroid tapering doses/duration nor further treatment plan for clinical complexity after receiving immunosuppressants is uncertain. This may be attributed to the lack of clinical data caused by premature death in these patients or the lack of consensus on the follow-up pathological mechanism and trends of CIP. Here, we support the longitudinal view of pathological development (23) to divide the pulmonary injury evolution process of CIP into three phases, from inflammation to fibrosis. This separate definition of the fibrotic phase in CIP out of usual pneumonitis grades (G1–G4) typing classification is crucial for treatment: immunosuppressives for inflammation and anti-fibrotics for fibrosis.

3.1.1 Acute inflammatory stage of CIP

Acute exaggerated inflammatory reactions in lung tissues contribute to the progressive reduction in pulmonary function, the primary cause of mortality in patients (69). Histologically, the acute phase of CIP is characterized by acute inflammatory and/or fibrinous exudation in the alveolar cavities (24, 55), accompanied by interstitial inflammatory infiltration and/or fibrosis, which is called mild organization formation (2, 31, 57). Interstitial inflammatory infiltration may include elevated levels of eosinophils, poorly formed granulomas, and lymphocytes (4, 69, 70).

3.1.1.1 Cytotoxic effects of T cells on instigation of CIP

The markedly increased percentage of lymphocytes infiltrating the malignant cells shows the superior tumor immune-clearance effects of ICIs (18). However, enhanced targeted T-cell activity can attack the cross-antigens shared between the tumor and normal lung tissues, leading to off-

target toxicity (2, 8, 71). Significant lymphocytosis enriched with CD4+ T cells (72) and CD8+ T cells (70, 73, 74) has been examined in the pulmonary tissues and BAL of patients with CIP, reflecting the participation of lymphocyte-mediated exaggerated immunological reactions. The CD4+ T-cell compartment showed an increase in the number of pathogenic T-helper 17.1 cells, while within the CD8+ T-cell compartment, the number of effector memory T cells were mainly increased (75). An increased proportion of activated autoimmune indicators, CD3+ T cells/HLA-DR + T cells, was associated with the severity grading of CIP (39). Malignant NSCLC tumor cells may have more cross-antigens than normal lung tissues, as they are incubated in the same pulmonary environment (76). Tumor antigens, autoantigens, and neoantigens released from cytotoxic T-lymphocyte-mediated cell lysis induced persistent amplification of immune responses through "epitope spreading" (77). T cells from tumor-infiltrating lymphocytes shared a notable overlap in receptor sequencing with the T cells infiltrating the inflammatory CIP lesions, but not with the secondary lymphoid organs or peripheral blood (78). In addition, decreased expression of PD-1 and CTLA-4 weakened the immune tolerance/anti-inflammatory function of the Treg population (79), whereas the number of central memory T cells with proinflammatory subsets increased (78), which contributes to tumor clearance and the inflammatory microenvironment.

3.1.1.2 Increased proinflammatory cytokines and autoantibodies

Laboratory plasma and BALF examinations of patients with NSCLC-CIP showed an increasing spectrum of common inflammatory cytokines, such as interleukin (IL)-1β, IL-6, IL-17A, IL-35, C-reactive protein, and procalcitonin (72, 76, 80, 81). The levels of surfactant protein-A, surfactant protein-D, and Krebs von den Lungen-6 (KL-6) produced by type II alveolar epithelial cells, which reflect alveolar epithelial cell injury, were also increased (75, 76, 82, 83). These biomarkers usually would decrease to normal levels when the initial respiratory symptoms are relieved. Notably, in addition to the known autoantibodies associated with autoimmune diseases with a higher incidence of CIP, some studies reported a nearly 1.34-fold increase in anti-CD74 plasma level (84) in patients with CIP, while CD74 was related to interstitial pneumonitis (85, 86).

3.1.2 Subacute and chronic phase of CIP: profibrotic and fibrotic stages

For most CIP patients with mild to moderate grades, inflammatory exudation would be absorbed efficiently, with complete weaning off of steroids after 6–8 weeks of initial treatment, leaving mild asymptomatic lesions in the lungs (39, 55). Higher severity and longer duration of inflammation in CIP might exert a great influence on the alveolar microenvironment (72). This could excessively trigger the body's wound-healing mechanisms (87), leading to persistent reparative attempts by the lung, which manifest as pneumocyte hyperplasia, alveolar epithelial hyperplasia, fibroblastic proliferation, fibrous tissue hyperplasia, alveolar septal thickening, interstitial fibrosis, and lymphocyte infiltration (9, 55, 58, 79). The absorption rate of the lesions is slow or unchanged over an extended period. Even when inflammation is gradually suppressed, activated, and amplified, fibrotic processes may not stop. Chronic CIP was clinically defined as the occurrence of persistent or worsened CIP after steroid taper and CIP recrudescence during steroid taper, which extended the course of immunosuppression to more than 12 weeks (23, 39, 55). During this stage, fibrosing interstitial lung diseases (such as entirely damaged normal lung structure, thickening, and occlusion of blood vessels) are dominant in histology, which might impair air–blood exchange efficiency in the lungs (2, 11, 23). The typical sequelae might be sustained pulmonary interstitial fibrosis and poor pulmonary function caused by severe CIP (39). These patients are more vulnerable to irreversible fibrosis under certain conditions that permanently deteriorate the pulmonary function. Anti-fibrotic agents are thus necessary for this condition.

3.1.2.1 Possible mechanisms in profibrotic and fibrotic stages

Multi-causal fibrotic lung injuries may have pathophysiological mechanisms that overlap with those of idiopathic pulmonary fibrosis, suggesting the potential to share common treatment approaches (59, 88, 89). Early treatment is crucial for slowing the decline in lung function and improving clinical outcomes.

Repetitive injury and reprogramming of the alveolar epithelium (59) are significant triggers of fibrosing progression, inducing an aberrant wound healing response that is characterized by proliferation of fibroblasts and myofibroblasts (61), extracellular matrix (ECM) remodeling (62), and subsequent loss of lung architecture and function (60, 88, 89). Activated fibroblasts and myofibroblasts can secrete ECM components, while myofibroblasts can produce contractile apparatus, such as α-smooth muscle actin microfilaments, which leads to the formation of fibroblastic foci and deposition of ECM (90). Fibroblastic foci in the alveolar parenchyma (91–93) and collagen expansion of the alveolar septa (94) have been detected in CIP lungs. These changes can destroy normal lung structures, causing a characteristic "honeycombing" morphology.

Profibrotic mediators, such as IL-1β (95), transforming growth factor (TGF)-β (96), sphingosine1-phosphate (S1P) (97), and WNT (98) ligands, are involved in this process. TGF-β can induce an unabated form of the fibrotic process called epithelial-to-mesenchymal transition (EMT) (99), in which alveolar epithelial type II cells (undergoing endoplasmic reticulum stress (100), mitochondrial dysfunction (101), or senescence (102) can serve as an unlimited source for the increased myofibroblast-like pool, if the primary inflammatory

insult is not attenuated. The microvascular pulmonary endothelium attached to the alveolar epithelium could also become another source of myofibroblasts by endothelial-to-mesenchymal transition (103), which potentially contributes to lung fibrosis and pulmonary hypertension. In addition, immune cells have been detected to infiltrate the chronic inflammatory (91, 93, 94) and fibrotic lesions of CIP. Mast cells (MCs) are innate immune cells that play a key role in the early response to tissue injury (104) and can release an array of profibrotic mediators (105–107), such as histamine, TGF-β (108), MC tryptase (109), and MC chymase (110). MCs would infiltrate immediately the surrounding fibroblast foci, forming a mutually excitatory MC–fibroblast crosstalk in fibrosis that activates the proliferation of MC and fibroblast (106). The number of MCs is positively correlated with the number of fibroblast foci, which has been linked to increased mortality.

3.1.2.2 Crosstalk between pulmonary fibrosis and lung cancer

One particularly special condition in patients with NSCLC-CIP is that both cancer development and immunotherapy adverse effects occur in the lungs. Tumor cells are not isolated but are in constant communication with other cell types within the lung tissue, which forms a particular microenvironment (111). Lung resident fibroblasts surrounding the malignancy are considered first responders to the site of insult that the tumor creates (112), while primary cancerous cells can also infiltrate the abnormal microenvironment that undergoes inflammatory and fibrotic processes of CIP, with the possibility of various cellular active substances and signaling molecules interacting with each other (113, 114).

As early as 1965, the crosstalk between lung cancer and pulmonary fibrosis has been suggested (115), which might pathologically exacerbate their progression (111, 113). The tumor stroma, fibrovascular networks, and tumor microenvironment are vital for the preservation and proliferation of the tumor and its structural integrity. Bronchiolar epithelial cells can undergo reversible EMT (116) during the early stages of carcinogenesis, cancer invasion, and metastasis. Cancer-associated fibroblasts (CAFs) are important players in lung cancer because they exhibit mesenchymal-like features and help build the structure of the tumor stroma (117) with heterogeneous phenotypes. The tumor vasculature can establish fibrovascular networks for the preparation of invasion. Endothelial-to-mesenchymal transition is thought to be the source of 40% of CAFs (118) and may play a role in tumor angiogenic sprouting into adjacent tissues. Through the action of platelet-derived growth factor-BB (PDGF-BB), vascular pericytes released by tumor microvasculature can differentiate into stromal fibroblasts (119), which significantly contributes to tumor invasion and metastasis. Profibrotic mediators, such as TGF-β, are also chronically overexpressed in lung cancer (112). Tyrosine kinase receptor ligands, such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF), are aberrantly expressed in lung cancer and IPF (114). For example, PDGF plays a critical role in stimulating the secretion of ECM components and growth factors, thereby promoting fibroblast proliferation and recruiting fibrocytes to the lungs.

3.2 Nintedanib

Nintedanib (development code: BIBF 1120) is a molecule that does not exist in nature and is a pure synthetic compound synthesized in 1998 during a study on small-molecule inhibitors of angiogenesis (25) [ATC Code: L01XE31 (120)]. Herein, we present the classic concepts and novel experimental results regarding the functional mechanisms of nintedanib to explore its potential efficacy in cancer patients with existing or secondary pulmonary fibrosis (such as CIP).

3.2.1 Mode of action

Receptor tyrosine kinases (RTKs) belong to a group of key enzymes that catalyze important cellular quality and processes, such as cell shape, cell motility, cell cycle control, functional differentiation, gene transcription, and synaptic transmission (121). Abnormal or excessive tyrosine phosphorylation is associated with various cancers (122, 123), inflammatory diseases (124, 125), and pulmonary fibrotic diseases, such as IPF and ILD (126). Therefore, drugs that antagonize related protein tyrosine kinases and phosphatases are expected to control these diseases.

Nintedanib is a small-molecule tyrosine kinase inhibitor that competitively binds to the ATP-binding pocket of kinase to block intracellular signaling cascades (Figure 7) (127). It potently inhibits the RTKs implicated in the pathogenesis of various diseases, including vascular endothelial growth factor receptors (VEGFRs), fibroblast growth factor receptors (FGFRs), and platelet-derived growth factor receptors (PDGFRs) (128, 129). Nintedanib also inhibits the receptor tyrosine kinase FLT-3 and non-receptor tyrosine kinases Lck, Lyn, and Src (128), although the contribution of inhibition of these kinases to the therapeutic activity of nintedanib has been less reported (130). In addition to competitively binding to ATP receptor pockets, nintedanib blocks protein kinase activity by allosterically modulating (131) the conformation of ATP-binding sites to inhibit phosphorylation (132). Therefore, nintedanib may exert its anti-angiogenesis, anti-fibrotic, anti-inflammatory, and anti-tumor functions by separately or simultaneously blocking these tyrosine kinases.

The VEGF family binds to VEGFR-1, VEGFR-2, and VEGFR-3 (133). It can promote endothelial cell proliferation and survival, increase vascular permeability, and regulate tumor-related angiogenesis, which is critical for tumor growth and metastasis (134, 135). In NSCLC, elevated VEGF/VEGFR

expression is associated with poor prognosis (136). Nintedanib inhibits the proliferation of three types of cells involved in angiogenesis by blocking VEGFRs: endothelial cells, pericytes, and smooth muscle cells (128, 137). There are four types of FGF receptors: FGFR-1, FGFR-2, FGFR-3, and FGFR-4 (138). FGFs play an important role in tissue repair, angiogenesis, inflammation, and tumor initiation and progression (139, 140). Studies have shown that blocking FGFRs can reduce alveolar interstitial fibrosis and inhibit proliferation, migration, and differentiation of fibroblasts to myofibroblasts (141, 142). FGFRs might be anti-tumor therapeutic targets, as FGFR-1 amplification occurs in approximately 20% of patients with SqCLC and is associated with a poor prognosis (143). The PDGF family interacts with homodimers or heterodimers of PDGFR-α/β (144). The PDGF/PDGFR pathway promotes the proliferation, survival, and migration primarily of cells of mesenchymal origin (142). It is associated with fibrosis, cancer proliferation, metastasis, invasion, and angiogenesis (145, 146). Together with FGFRs, PDGFRs regulate the migration and adhesion of pericytes and the transformation of smooth muscle cells to endothelial cells, providing support and stability to vascular walls (144). Nintedanib could reduce the number of blood platelets, block the differentiation of fibroblasts to myofibroblasts, inhibit EMT, and suppress inflammation and angiogenesis by inhibiting these receptors (133, 147).

Pulmonary interstitial inflammatory infiltration in fibrotic lesions may include elevated levels of eosinophils, poorly formed granulomas, and lymphocytes (4). Nintedanib significantly reduced the infiltration of immune cells (including mast cells [MCs] and eosinophils) and neutrophils (147), but not that of macrophages, inhibited granuloma formation (148), and decreased the levels of other proinflammatory cytokines, including IL-4, IL-5, IL-6, and IL-13 (149), during lung fibrosis. Notably, the significant reduction in lymphocyte count by nintedanib was dependent on the early initiation of treatment (150). As mentioned in *Section 3.1.2*, the number of MCs was increased in the region immediately surrounding the fibroblast foci, activating crosstalk with fibroblasts in fibrosis (106). Nintedanib could inhibit recombinant stem cell factor-induced MC survival by directly blocking the phosphorylation of stem cell factor-stimulated c-kit (a type of tyrosine kinase receptor also called stem cell factor receptor) and could reduce the infiltration of MCs (147). Nintedanib also significantly reduced the secretion of TIMP-1, IL-1β, and TGF-β, factors that play key roles in the proinflammatory and profibrotic pathways of pulmonary fibrotic changes (40). Nintedanib reduced pulmonary fibrosis by inhibiting growth-factor-induced proliferation (151) and motility (150) of lung fibroblasts, TGF-β-induced transformation of fibroblasts to myofibroblasts, EMT with increased E-cadherin levels (152),

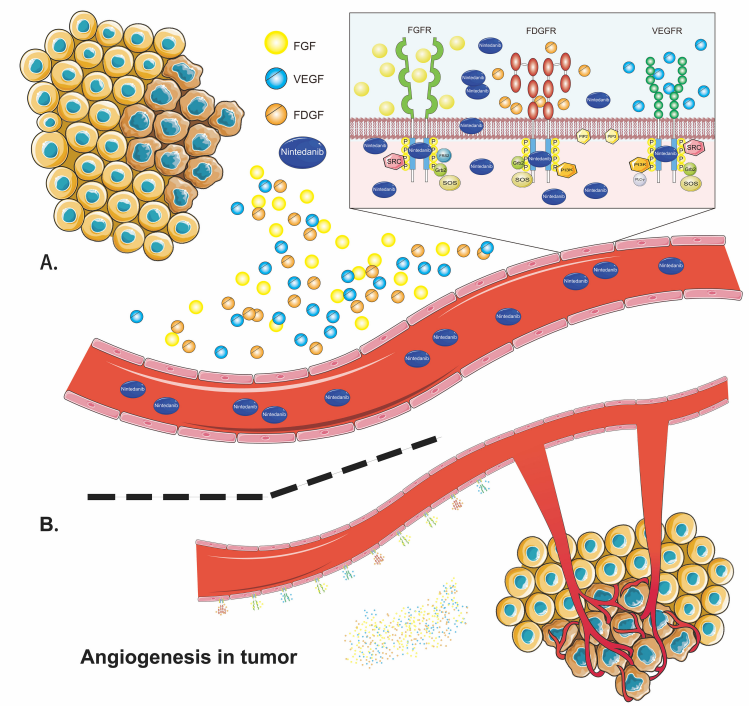

FIGURE 7
The basic mechanism of nintedanib.

and ECM collagen secretion and ECM deposition (40), and reducing fibrotic gene expression (including collagen 1a1 and fibronectin) (153). Repetitive injury and reprogramming of the lung epithelium are considered critical drivers of fibrosis progression. Nintedanib increased the expression of the transcription factor Nkx2.1 in isolated ATII cells and stabilized the expression of distal lung epithelial cell markers, especially SP-C, to restore normal alveolar epithelial cell function that contributes to its anti-fibrotic effects (153).

Only a few studies have explored the antitumor mechanism of nintedanib. Nintedanib notably increased the ratio of α-smooth muscle actin+/CD31+, a sign of tumor vessel normalization, and reduced distorted vessel density in tumors (45). It also suppressed tumor proliferation by inhibiting CAFs (46, 154) and may inhibit tumor metastasis by blocking EMT (147). Moreover, recent evidence suggests that nintedanib can boost the efficacy of immunotherapy by upregulating MHC-I and PD-L1 expression and increasing the infiltration of $CD8^+$ T cells and DC cell maturation to enhance tumor sensitivity to immunotherapy in both *in vivo* and *in vitro* experiments (45, 46), which warrants further clinical evaluation.

3.2.2 Therapeutic efficacy and safety

As a triple angiokinase inhibitor, nintedanib has been clinically studied for pulmonary fibrosis and solid tumors. It is one of the only two drugs currently available to treat IPF (155) and is the only drug approved for use in patients with other progressive fibrosing ILDs and SSc-ILD (156). Nintedanib, in combination with docetaxel, has also been approved by the European Union (30) for the treatment of patients with advanced adenocarcinoma NSCLC. Herein, we summarize previous clinical and experimental evidence of the anti-fibrotic and anti-tumor therapeutic efficacy of nintedanib.

3.2.2.1 In pulmonary fibrosis: potent anti-fibrotic efficacy

Anti-fibrotic drugs, although not curative, can slow ILD progression and have facilitated a paradigm shift in the treatment of IPF over the last decade (150). Nintedanib has been shown to have anti-fibrotic and anti-inflammatory effects in animal models (148) and has shown potent anti-fibrotic effects in well-designed clinical trials of IPF and ILDs (27). It significantly reduced forced vital capacity decline and prolonged the time to first acute exacerbation in patients with IPF (157), irrespective of existing physiological impairment, persistent positive anti-fibrotic effects to slow down IPF progression over more than 4 years of treatment in the INPULSIS trial (42), and improved OS and PFS in the EMPIRE registry (158). Nintedanib has achieved similar effects in reducing annual rates of forced vital capacity decline in patients with chronic fibrosing pulmonary interstitial diseases with a progressive phenotype (such as chronic hypersensitivity pneumonitis and autoimmune ILDs) in the INBUILD (40, 159) and SENSCIS trials (26). Nintedanib has also been shown to reduce lung function deterioration in patients with other fibrotic patterns to reduce lung function deterioration (28, 27, 160).

3.2.2.2 In cancer: Positive anti-tumor efficacy

Nintedanib has shown positive anti-tumor effects in clinical trials on esophageal (161), colon (162), breast (163), prostate (164), and lung cancers, thus attracting increased attention from researchers. Modest disease stabilization was observed in patients undergoing metastatic esophagogastric treatment with nintedanib (161). Nintedanib modulates tumor blood flow and permeability in patients with advanced refractory colorectal cancer (162). In combination with standard chemotherapy, a full dose of nintedanib achieved a 50% pathological complete response in early HER-2-negative breast cancer (163). Nintedanib in combination with afatinib (BIBW 2992), an ErbB family blocker, has shown limited antitumor activity in patients with advanced castration-resistant prostate cancer (164). Combined nintedanib plus bevacizumab treatment achieved a disease control rate of 72.2% in patients with solid tumors (lung, colon, and cervical), even in those with bevacizumab resistance (165).

According to clinical trials of patients with lung cancer, nintedanib monotherapy exhibited relatively inferior efficacy compared to combination therapy (Table 2). In 2011, Reck et al. studied the efficacy of nintedanib monotherapy for patients with advanced NSCLC (172). They reported an mPFS of 6.9 weeks, mOS of 21.9 weeks, and disease control rate (DCR) of 49.3% (172). Nintedanib monotherapy has also shown limited activity in relapsed SCLC (173). In later trials, the combination of nintedanib plus pemetrexed achieved a better DCR ranging from 60.9% to 66.7% in patients with advanced recurrent NSCLC (44, 166, 167). Doebele et al. tested nintedanib, in combination with paclitaxel plus carboplatin, in chemotherapy-naive patients with advanced NSCLC and observed a DCR of 84.6% (168). For patients with advanced SqCLC, the LUME-Lung 3 study reported a DCR of 81.3% under first-line combination therapy of nintedanib plus cisplatin plus gemcitabine, with an mPFS of 4.2 months and an mOS of 6.7 months (169). In lung adenocarcinoma, combined nintedanib and docetaxel therapy has been approved as first-line chemotherapy in the European Union (30). The DCR in trials of this combination after first-line chemotherapy ranged from 54.7% to 72.7%, with the longest mPFS and mOS of 5.7 and 12.6 months, respectively (43, 170, 171, 174–176). Three non-interventional studies reported the efficacy of nintedanib and docetaxel after chemo-immunotherapy with the DCR of 78.2%–86% (29, 177, 178). Nintedfanib has shown potent antitumor efficacy combined with chemotherapeutics in patients with NSCLC after first-line chemotherapy or chemo-immunotherapy. As most of these trials were conducted in European countries, more clinical evidence from other regions is warranted.

3.2.2.3 In patients with NSCLC-CIP: Potential of dual anti-fibrotic and anti-tumor efficacy

Whereas baseline pulmonary fibrosis diseases, such as IPF and ILD, are considered to limit ICI application and severely impair the survival outcomes of patients with NSCLC, nintedanib therapy is a valuable strategy for patients with cancer, complicated with preexisting pulmonary fibrosis. Fukunaga et al. reported that nintedanib inhibited the growth of a newly discovered nodule for 9 months during AE-IPF treatment; the tumor started to increase in size 4 months after the cessation of nintedanib, and the patient was diagnosed with squamous cell carcinoma (41). Nintedanib can exert dual anti-fibrotic and anti-tumor effects in patients with NSCLC with baseline pulmonary fibrosis. Shiratori et al. recently reported that nintedanib monotherapy successfully relieved pulmonary fibrosis and achieved remission of the primary tumor and pleural disseminations in an elderly patient with NSCLC complicated by IPF when he could not tolerate cytotoxic chemotherapy due to IPF progression (179).

According to immunotherapy, a significantly higher incidence rate (10) of any grade of CIP has been reported in patients with NSCLC with preexisting ILD. Unspecified baseline fibrosis was the strongest independent predictor of CIP in patients with NSCLC (11), compared with pertinent demographics and PD-L1 expression. Risk factors for chronic pulmonary fibrosis diseases (180) considerably overlap with those for lung cancer, which may account for the increased burden of CIP in lung cancer patients with preexisting pulmonary fibrosis. However, some of these overlapping risk markers, such as smoking history, are associated with a better clinical outcome in patients with NSCLC treated with ICIs (181); this may explain the favorable clinical overall response rate of immunotherapy observed in patients with NSCLC with preexisting ILD (182–186), as most had a history of smoking. Another meta-analysis that included 10 East Asian studies pooled a significantly higher overall response rate and DCR after PD-(L)-1 inhibitor treatment in patients with preexisting ILD than in those without ILD (10). The reported favorable efficacy might sway the common belief that preexisting pulmonary fibrosis is a contraindication for immunotherapy. It might be an appropriate choice to combine nintedanib with immunotherapy for patients with preexisting pulmonary fibrosis.

In addition, the occurrence of CIP many be strongly correlated with a better tumor remission outcome, as mentioned previously at the beginning of *Section 3*. Beyond baseline fibrosis, patients with NSCLC with a smoking history, older age, squamous cell carcinoma subtype, and pre-existing pulmonary diseases, and those using PD-1 inhibitor were more likely to be at risk of CIP. Thus, patients with a higher risk of developing CIP, especially those with preexisting pulmonary fibrosis, should be closely monitored during ICI therapy or after ICI termination and should be given early diagnosis and intervention under a multidisciplinary approach to obtain the best clinical efficacy from ICIs. Notably, CIP may develop months after ICIs discontinuation (187). Nintedanib could slow down the decline rate of forced vital capacity (40) and potentially strengthen the prevention of CIP (188) when used in combination with ICIs or after ICIs in cancer patients.

Beyond its potential to alleviate pulmonary fibrosis and overcome ICI side effects, nintedanib can increase the efficacy of immunotherapy and overcome ICI resistance (46, 45). The synergistic effect of nintedanib and anti-PD-1 therapy on lung cancers was revealed in *in vitro* and *in vivo* experiments (45); significantly increased ICI therapy responses, relieved aggravated lung injuries, inhibited tumor metastasis, and activated tumor immune microenvironment were observed (46). Nintedanib upregulated the expression of PD-L1 and MHC-I in tumor cells, boosted the immunological recognition and immune clearance of tumor cells, and increased the infiltration and activation of immune cells in tumor tissues, thus improving the efficacy of immunotherapy and overcoming partial ICI resistance (45). In addition, it significantly reduced pulmonary fibrosis in the nintedanib+anti-PD-L1 group, whereas evident pulmonary consolidation was observed in the control and anti-PD-L1 groups (45). These results suggest the dual potential of nintedanib to boost the tumor clearance effects of immunotherapy and prevent or alleviate the development of CIP when applied in combination with ICIs.

A current monocentric phase Ib cohort that studied the safety and efficacy of nintedanib in combination with pembrolizumab in patients with advanced solid tumors reported no case of CIP after combined treatment (189), and nintedanib increased Treg recruitment and circulation of helper memory T cells in the tumor microenvironment to enhance the tumoral immune response (189). These findings confirm the observed antitumor efficacy of nintedanib independent of angiokinase activity (130), which warrants further investigations to explore the antitumor usage of nintedanib in combination with immunotherapy.

Pharmacokinetic studies have shown that nintedanib is rapidly absorbed after oral administration. The pharmacokinetics of nintedanib are time independent and dose linear (172). In patients with advanced cancer, twice-daily administration could achieve a stable state for up to 7 days (127, 190), with a terminal half-life of 7–19 h (129). The cumulative effect of repeated administration was negligible (190). However, current studies have demonstrated that nintedanib can act against a wide range of diseases; however, its low bioavailability is a future challenge for maximizing its effects (191).

In clinical trials, nintedanib has a controlled safety profile, in combination with docetaxel (192), pemetrexed (166), paclitaxel/carboplatin (168), and afatinib (193), with a maximum tolerated dose of 200 mg B.I.D. Blockade of VEGF leads to decreased platelet activity and decreased leukocyte adhesion (194). It has anticoagulant effects and increases the risk of bleeding and thrombosis (165). In addition, blocking PDGF-α and PDGF-β can lead to thrombocytopenia by affecting platelet production (161). Adverse events associated with anti-angiogenic agents in

TABLE 2 From phase I to phase III clinical trials on nintedanib for lung cancers.

Clinical trial (phase)	Reference (details in the annotation)	Year	Patient characteristics	After chemo or chemoimmunotherapy	Patients (n)	Drug combination	Comparator	N dose/ frequency	Response n(%)			Disease control	mPFS (mo)	mOS (mo)
									Complete response	Partial response	Stable disease			
I	Ellis et al. (166)	2010	Recurrent NSCLC	previously treated with one first-line platinum-based chemotherapy regimen	26	Pemetrexed + BIBF 1120 (Former Nintedanib)	–	100 or 150 or 200 or 250 mg/ bid	1(0.04%)	4(15.4%)	13(50%)	17 (65.3%)	5.4	NA
I	Daga et al. (167)	2015	Stage IIIB/IV or recurrent NSCLC	after or failure of prior first-line chemotherapy	18	Pemetrexed + Nintedanib	–	100 or 150 or 200 mg/bid	0	2(11.1%)	10 (55.6%)	12 (66.7%)	NA	NA
I	Doebele et al. (168)	2012	Chemotherapy-naive advanced NSCLC	as first-line treatment	26	Paclitaxel + carboplatin + Nintedanib	–	50 mg/bid	0	7(26.9%)	15 (57.7%)	22 (84.6%)	NA	NA
I	LUME-Lung 3 study (169)	2018	Advanced sqNSCLC	as first-line treatment	4+12	Cisplatin + Gemcitabine + Nintedanib (150mg bid)	Cisplatin + Gemcitabine + Nintedanib (200mg bid)	150 or 200 mg/bid	0	5(31.3%)	8(50%)	13 (81.3%)	4.2	6.7
I	Okamoto et al. (170)	2015	Stage IIIB/IV or recurrent NSCLC	had received one platinum-based chemotherapy regimen (not containing docetaxel)	38	Docetaxel + Nintedanib	–	100 or 150 or 200 mg/bid	0	10 (26.3%)	18 (47.3%)	28 (73.7%)	5.7	NA
Ib	Yamamoto et al. (171)	2018	Lung adenocarcinoma	after the failure of first-line platinum-based chemotherapy	10	Docetaxel + Nintedanib	–	200 mg/ bid	0	4(40%)	3(30%)	7(70%)	NA	NA
II	Reck et al. (172)	2011	Stage IIIB/IV or recurrent NSCLC	after chemotherapy (including one platinum-based chemotherapy)	73	BIBF 1120 (Former Nintedanib)	–	150 or 250 mg/bid	0	1(1.4%)	35 (47.9%)	36 (49.3%)	6.9w	21.9w
II	Youn Han et al. (173)	2016	relapsed/refractory SCLC	during or after treatment with at least one platinum-based chemotherapy	22	Nintedanib	–	200 mg/ bid	0	1(0.05%)	7(31.8%)	8(36%)	1	9.8
II	REFRACT GFPC 02-15 study (174)	2021	Advanced NsqNSCLC	documented progression during first-line chemotherapy based on a platinum-doublet and third- generation drug for ≤ 4 cycles	53	Docetaxel + Nintedanib	–	200 mg/ bid	0	10 (18.9%)	19 (35.8%)	29 (54.7%)	2.7	6.9
IIb	SENECA trial (175)	2019	Recurrent NsqNSCLC	received one previous chemotherapy regimen	85+85	Docetaxel (33mg/mq) + Nintedanib	Docetaxel (75mg/mq) + Nintedanib	200 mg/ bid	NA	NA	NA	107 (63.0%) vs. 124 (72.7%)	4.79 vs. 4.82	8.49 vs. 9.62
III	LUME-Lung 1 study (43)	2014	Stage IIIB/IV or recurrent NSCLC	after first-line chemotherapy	655 +659	Docetaxel + Nintedanib	Docetaxel + Placebo	200 mg/ bid	0 vs. 1 (0.2%)	29(4.4%) vs. 21 (3.2%)	325 (49.6%) vs. 250 (37.9%)	354 (54.0%) vs. 272 (41.3%)	3.4 vs. 2.7	10.1 vs. 9.1
III	LUME-Lung 1 study (43)	2014	Stage IIIB/IV or recurrent lung adenocarcinoma	after first-line chemotherapy	332 +336	Docetaxel + Nintedanib	Docetaxel + Placebo	200 mg/ bid	0 vs. 0	15(4.7%) vs. 12 (3.6%)	179 (55.6%) vs. 136 (40.5%)	194 (60.2%) vs. 148 (44.0%)	4.2 vs. 1.5	12.6 vs. 10.3

(Continued)

10.3389/fimmu.2022.1072612

TABLE 2 Continued

Clinical trial (phase)	Reference (details in the annotation)	Year	Patient characteristics	After chemo or chemoimmunotherapy	Patients (n)	Drug combination	Comparator	N dose/ frequency	Response n(%)			Disease control	mPFS (mo)	mOS (mo)
									Complete response	Partial response	Stable disease			
III	LUME-Lung 2 study (44)	2016	Stage IIIB/IV or recurrent NsqNSCLC	had received one prior chemotherapy	353 +360	Pemetrexed + Nintedanib	Pemetrexed + Placebo	200 mg/ bid	0	32(9.1%) vs. 30 (8.3%)	183 (51.8%) vs. 162 (45.0%)	215 (60.9%) vs. 192 (53.3%)	4.4 vs. 3.6	12.0 vs. 12.7
III	LUME-Lung 2 study (44)	2016	Stage IIIB/IV or recurrent lung adenocarcinoma	had received one prior chemotherapy	335 +335	Pemetrexed + Nintedanib	Pemetrexed + Placebo	200 mg/ bid	0	32(9.1%) vs. 30 (8.3%)	175 (52.2%) vs. 153 (45.7%)	207 (61.8%) vs. 183 (54.6%)	4.5 vs. 3.9	12.3 vs. 13.1
III	Cid JR et al. (176)	2016	Advanced lung adenocarcinoma	NA	99	Docetaxel + Nintedanib	–	200 mg/ bid	0	27 (27.2%)	52 (52.5%)	79 (79.6%)	NA	NA
*Non-interventional study	Corral et al. (29)	2019	Advanced lung adenocarcinoma after chemotherapy and immunotherapy	After chemo-immunotherapy	11	Docetaxel + Nintedanib	–	200mg/bid	0	4(36%)	5(46%)	9(82%)	3.2	NA
*Non-interventional study	VARGADO (177)	2022	Advanced lung adenocarcinoma after chemotherapy and immunotherapy	After chemo-immunotherapy	80	Docetaxel + Nintedanib	–	200mg/bid	1(1.6%)	31 (48.4%)	23 (35.9%)	55(86%) (total=64)	6.4	12.1
*Non-interventional study	LUME-BioNIS study (178)	2020	Advanced lung adenocarcinoma after chemotherapy and immunotherapy	After chemo-immunotherapy	55	Docetaxel + Nintedanib	–	200mg/bid	0	10 (18.2%)	33(60%)	43 (78.2%)	4.6	8.8

10.3389/fimmu.2022.1072612

cancer therapy include thromboembolic events, gastrointestinal perforation, bleeding, hypertension, and proteinuria (194). In SqCLCs, bevacizumab (a monoclonal antibody that targets the VEGFR ligand) is not recommended because of the increased risk of severe or even fatal pulmonary hemorrhage (195). Other anti-VEGF small-molecule TKIs, sorafenib and sunitinib, can induce skin-associated adverse events (135), and sorafenib, in combination with carboplatin and paclitaxel, increases the risk of death (196).

Nintedanib is an antiangiogenic agent with an acceptable safety profile and good tolerance in patients with cancer (174). In clinical trials, gastrointestinal reactions, such as mild nausea or vomiting, were the most common adverse events associated with nintedanib (189, 192). Although nintedanib blocks VEGF and PDGF receptors, resulting in a potentially increased risk of bleeding, there is no increased incidence of cardiovascular or bleeding complications observed in large clinical trials involving nintedanib (43, 157, 175). It should be noted that clinical trials have excluded patients with a bleeding tendency during enrollment, which might have resulted in biased results. Moreover, a post-marketing data review showed that <5% of 6,758 patients treated with nintedanib experienced adverse bleeding events, and <1% of patients experienced major bleeding events for approximately 1 year (30). Bleeding events most often involve the digestive, respiratory, and central nervous systems (42). Mild epistaxis was the most common bleeding event (177).

The most common drug-related adverse reactions in patients with advanced NSCLC treated with nintedanib monotherapy were nausea (57.5%), diarrhea (47.9%), vomiting (42.5%), anorexia (28.8%), abdominal pain (13.7%), and reversible elevation of alanine aminotransferase (13.7%) and aspartate aminotransferase (9.6%) levels (172). In the LUME-BioNIS study, the most common on-treatment ADRs/adverse events of nintedanib plus docetaxel were diarrhea (32.3%), malignant neoplasm progression (29.2%), and nausea (15.4%) (178). A phase II study of nintedanib therapy in relapsing small cell lung cancer identified elevated AST/ALT levels and neutropenia as major causes of treatment delay and subsequent dose adjustment (173).

In the present case, the elderly patient showed good tolerance to nintedanib monotherapy (200 mg B.I.D) for long-term treatment and had no adverse hemorrhagic reactions, except for slight dizziness and nausea after nintedanib initiation, which improved later.

3.2.3 Potential of nintedanib in patients with NSCLC with G3-G4 CIP

3.2.3.1 Dual management advantages in critical stage of CIP with good tolerance

Patients who experienced deteriorated or maintained CIP were significantly more likely to have a poor prognosis than those who experienced improved or resolved CIP (9, 11). Those who experienced G3–G4 CIP had to quit ICI therapy to avoid lethal respiratory failure (20), and their physicians tended to hesitate or delay commencement or continuation of aggressive anti-tumor treatment owing to deteriorating physical status, abrasive pulmonary symptoms, and prolonged immunosuppressive management for CIP. Moreover, some immunosuppressive agents, such as infliximab, might weaken the ongoing antitumor immune activity initially launched by ICI treatment (22) during this critical stage.

Nintedanib, with its good safety profile, may be a potent candidate to fill this gap by inhibiting tumor growth under critical conditions and alleviating potential pulmonary fibrosis caused by CIP pathogenesis. However, its efficacy and safety in CIP treatment have only been reported in one case. Xie et al. reported a case of CIP caused by pembrolizumab in a patient with NSCLC (adenocarcinoma) who was successfully treated with nintedanib. However, the patient experienced rapid tumor progression after 2 weeks of nintedanib monotherapy and died 2 months later (83). The patient in this study also showed significant tumor progression after 25 weeks of monotherapy with nintedanib, which must be controlled with other combination therapies.

3.2.3.2 Combination with other anti-tumor therapies in NSCLC

Long-term nintedanib monotherapy was not sufficiently effective to inhibit tumor progression in patients with NSCLC-CIP; however, it significantly ameliorated pulmonary fibrosis, reduced pulmonary function decline, and restored physical activity levels. Thus, physicians should combine other antitumor therapies with nintedanib after physical strength recovery. Here, we chose afatinib as second-line therapy, in conjunction with albumin–paclitaxel chemotherapy, leading to stabilized tumor progression with good tolerance of this case.

Afatinib is a broad-spectrum irreversible blocker of the ErbB receptor family that inhibits the EGFR (ErbB-1/HER1) signaling pathway, ErbB-2/neu/HER2, ErbB-3/HER3, and ErbB-4/HER4 (197). Afatinib has a broader inhibition spectrum than first-generation reversible EGFR-specific drugs, such as erlotinib and gefitinib (198). Although mutations in EGFR/ErbB-1/HER1 have rarely been identified, this pathway has been reported to play a role in the pathophysiology of SqCLC (199). Afatinib can be used as first-line treatment for patients with EGFR mutation-positive NSCLC and has shown considerable efficacy (200). However, given the scarcity of EGFR mutations (<4%) in SqCLCs (201, 202), most patients with advanced SqCLCs do not receive EGFR-TKIs, resulting in paucity of relevant clinical data. Compared with those of first-generation EGFR-TKIs (erlotinib/gefitinib), preclinical data of afatinib showed a lower IC50 value, greater potency capacity against wild-type EGFR antibodies, and the ability to inhibit ErbB-2/neu/HER2 (203–205).

EGFR/ErbB-1/HER1 overexpression occurs in >50% of NSCLCs, especially in >80% of SqCLC tissues (206, 207). The gene copy number of EGFR increases in >25% of patients with SqCLC (199). In addition to EGFR, other ErbB-2/neu/HER2 and

ErbB-3/HER3 proteins are overexpressed in more than 20% of SqCLC patients (208, 209). These indicate the potential efficacy of afatinib in mutation-negative patients with SqCLC. The LUX-Lung 8 study reported that the efficacy of afatinib in squamous NSCLC is independent of EGFR mutations (210). Two other phase II studies also showed that afatinib was effective in patients with advanced NSCLC with wild-type EGFR (211).

4 Conclusion

Severe-grade (with or without steroid-refractory) CIP is particularly worrisome and is potentially lethal in patients with NSCLC. Sufficient immunomodulator therapy to suppress progressive respiratory symptoms could trigger secondary infectious pneumonia and further deteriorate pulmonary function during CIP treatment. The MICU successfully rescued the case patient from danger. Pulmonary fibrosis, a key stage in the pathological evolution of CIP, warrants the use of anti-fibrotic agents beyond immunosuppressive agents for CIP. Nintedanib, a triple tyrosine kinase inhibitor mostly applied in ILDs, IPF, and NSCLC-adenocarcinoma, has shown potent anti-fibrotic efficacy in the case patient with severe steroid-refractory CIP; however, it has insufficient long-term anti-tumor efficacy by monotherapy and thus requires combination with other anti-tumor therapies to boost efficacy. The dual anti-fibrotic and anti-tumor effects of nintedanib on patients with NSCLC with CIP are worthy of further investigation with clinical trials. It might be a good supportive choice for patients with NSCLC and severe CIP to inhibit tumor and fibrosis progression when they cannot tolerate further toxic anti-tumor therapy.

Ethics statement

Written informed consent was obtained from the patient for publication of this case report and any accompanying data and images.

Author contributions

LP and FM: conceptualization, methodology, writing-editing, data curation, and writing—original draft preparation, visualization. WW: supervision and writing—reviewing. X-hW, HS, and PB: visualization, Investigation. JK: supervision, validation. D-lK: supervision and writing—reviewing and editing. All authors contributed to the article and approved the submitted version.

Acknowledgments

We would like to thank the Respiratory and Critical Care Medicine Department of the First Hospital of China Medical University for the great healthcare services and warm supports for this paper especially in the crisis of the COVID-19.

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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10.3389/fimmu.2022.1072612

Glossary

NSCLC	non-small cell lung cancer
CIP	checkpoint inhibitor-related pneumonitis
irAEs	immune-related adverse events
PD-1	programmed cell death-1
PD-L1	programmed cell death ligand-1
CTLA-4	cytotoxic T-lymphocyte antigen 4
PFS	progression-free survival
OS	overall survival
FAEs	fatal adverse events
MICU	medical intensive care unit
IPF	idiopathic pulmonary fibrosis
ILDs	interstitial lung diseases
SqCLC	squamous cell lung carcinoma
NGS	next-generation sequencing
ECOG	Eastern Cooperative Oncology Group
GGOs	ground-glass opacities
PR	partial response
G1–4	grades 1–4
BALF	bronchoalveolar lavage fluid
IVIG	intravenous immunoglobulin
OP	organizing pneumonia
PD	progressive disease
TIME	tumor immune microenvironment
COPD	chronic obstructive pulmonary disease
AIP	acute interstitial pneumonia
ARDS	acute respiratory distress syndrome
DAD	diffuse alveolar damage
HP	hypersensitivity pneumonitis
NSIP	nonspecific interstitial pneumonia
CRP	C-reactive protein
PCT	procalcitonin
SP-A	surface protein-A
ECM	extracellular matrix
α-SMA	α-smooth muscle actin
TGF	transforming growth factor
S1P	sphingosine1-phosphate
EMT	pithelial-to-mesenchymal transition
AT	alveolar epithelial type
EnMT	endothelial-to-mesenchymal transition
MCs	mast cells
CAFs	cancer-associated fibroblasts
PDGF	platelet-derived growth factor
VEGF	vascular endothelial growth factor
FGF	fibroblast growth factor
RTKs	receptor tyrosine kinases
VEGFRs	vascular endothelial growth factor receptors
FGFRs	fibroblast growth factor receptors
PDGFRs	platelet-derived growth factor receptors
SCF	stem cell factor
FVC	forced vital capacity
MTD	maximum tolerated dose
AE	adverse events

frontiers | Frontiers in Oncology

TYPE Original Research
PUBLISHED 19 January 2023
DOI 10.3389/fonc.2022.1048127

OPEN ACCESS

EDITED BY
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ESSA Pharma Inc., United States

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SPECIALTY SECTION
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Oncology

RECEIVED 19 September 2022
ACCEPTED 26 December 2022
PUBLISHED 19 January 2023

CITATION
Guo M, Lu Z and Xiong Y (2023) Enhancer RNA-based modeling of adverse events and objective responses of cancer immunotherapy reveals associated key enhancers and target genes.
Front. Oncol. 12:1048127.
doi: 10.3389/fonc.2022.1048127

Enhancer RNA-based modeling of adverse events and objective responses of cancer immunotherapy reveals associated key enhancers and target genes

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Immune checkpoint inhibitors (ICI) targeting PD-1/PD-L1 or CTLA-4 are emerging and effective immunotherapy strategies. However, ICI-treated patients present heterogeneous responses and adverse events, thus demanding effective ways to assess benefit over risk before treatment. Here, by integrating pan-cancer clinical and molecular data, we tried to predict immune-related adverse events (irAEs, risk) and objective response rates (ORRs, benefit) based on enhancer RNAs (eRNAs) expression among patients receiving anti-PD-1/PD-L1 therapies. We built two tri-variate (eRNAs) regression models, one (with ENSR00000326714, ENSR00000148786, and ENSR00000005553) explaining 71% variance (R=0.84) of irAEs and the other (with ENSR00000164478, ENSR00000035913, and ENSR00000167231) explaining 79% (R=0.89) of ORRs. Interestingly, target genes of irAE-related enhancers, including upstream regulators of MYC, were involved in metabolism, inflammation, and immune activation, while ORR-related enhancers target *PAK2* and *DLG1* which participate in T cell activation. More importantly, we found that ENSR00000148786 probably enhanced TMEM43/LUMA expression mainly in B cells to induce irAEs in ICI-treated patients. Our study provides references for the identification of immunotherapy-related biomarkers and potential therapeutic targets during immunotherapy.

KEYWORDS

enhancer RNA (eRNA), immune checkpoint block therapy, adverse effect, drug responses, TCGA, pan-cancer analysis, TMEM43/LUMA

Introduction

Immune checkpoints (ICs) generally refer to key inhibitory factors of the immune system, including programmed cell death 1 (PD-1 or CD279) and its ligand programmed cell death 1 ligand 1 (PD-L1 or CD274) that control the T cell response and fate during tumor immunity (1). In tumor samples, PD-1 and PD-L1 mainly expressed in T cells and tumor cells, respectively, and tumors exploit their interaction to escape the immune system by counteracting the stimulatory signals from the interaction between T cell receptor (TCR) and major histocompatibility complex (MHC) and other costimulatory signals (2–4).

PD-1/PD-L1 has been translated to the clinical practice, and ICI treatment targeting PD-1/PD-L1 proved to offer significant clinical benefits in many cancers, with an ORR from 20% to 50% in multiple clinical trials and for various types of cancer (5). However, only a small subset of patients showed long-lasting remission, despite remarkable benefits of ICI therapies. Patients of some cancers were completely refractory to checkpoint blockade, occasionally leading to considerable side effects. To predict treatment benefit, PD-L1 expression was proposed as the first biomarker of anti–PD-1/PD-L1 therapy effectiveness (6), followed by tumor mutational burden (TMB) (7). Later, microsatellite instability (MSI) (8), CD8+ T-cell abundance (9, 10), cytolytic activity (11), and intestinal microbial composition (12) were proposed to prioritize patients with potentially more treatment gains.

On the other hand, irAEs result from excessive immunity against normal organs. Most studies show that the incidence of irAEs caused by anti-PD-1/PD-L1 treatment is about 60% (13, 14). Although nearly all organs can be affected, irAEs mostly involved the gastrointestinal tract, endocrine glands, skin, and liver (15). In some cases, irAE can be lethal. For example, pneumonitis is the most common fatal irAE with a 10% death rate, accounting for 35% of anti-PD-1/PD-L1-related fatalities (16). The mortality of myocarditis, the most lethal irAE, could even reach about 50% (17). Therefore, it is important and urgent to select patients with potentially significant benefit over risk of ICI treatments based on individual molecular data.

Although people have discovered several predictors of irAEs using expression of protein-coding genes (18), studying irAE-related non-coding elements would probably provide a better mechanistic understanding of why PD-1/PD-L1 pathway modulation leads to significant clinical benefit in some patients but temporary, partial, or no clinical benefit in other patients.

Recent studies found that eRNAs (non-coding RNAs) were usually transcribed from active enhancers and eRNA levels represent enhancer activities across tissues (19). Numerous cancer-associated eRNAs have been identified and eRNAs were proposed as potential therapeutic targets (20). Here, we comprehensively investigate the adverse events and the response rates in patients receiving anti-PD-1/PD-L1 therapies across cancer types. By integrating clinical data and molecular data, we identified three eRNAs for predicting irAE and another three eRNAs for ORR. Further exploring enhancer-target interaction identified functional genes that may help explain the overall risk or benefit of anti-PD-1/PD-L1 therapy, including MLXIPL, RAF1, MPL, PAK2, DLG1. In summary, our study reveals potential mechanisms underlying ICI therapy based on enhancer activity.

Results

Three eRNAs effectively predict irAE of immunotherapy

To identify factors to predict irAEs, we first examined correlations between 7 045 eRNAs and irAE RORs across 25 cancer types. ENSR00000041252 showed the highest correlation (correlation R=0.68, P=1.6e-4; Figure S1A), stronger than immune factors, including naive B cells, CD8+ T cells, macrophages M1, and T cell receptor diversity (18).

Then, we selected the top ten eRNAs with positive correlation and nominal significance (P<0.05) (Table S1, see **Methods**) to build prediction models, following a step-by-step procedure (Figure 1A). Multicollinearity analysis resulted in six roughly independent eRNAs, ENSR00000041252, ENSR00000326714, ENSR00000148786, X14.65054944.65060944, ENSR0000118775, and ENSR00000242410 (Figure 1B, 1C). Next, we obtained 15 significant bivariate regression models using the irAE-correlated enhancers. Correlation between the observed and predicted irAE ROR values showed that two eRNA combinations, ENSR00000148786 + ENSR00000005553 and ENSR00000148786 + ENSR00000251495, achieved the best predictive performance (R=0.79, P=3.1e-6; Figure S1B). Further increasing model factors resulted in the optimal tri-variate model, ENSR00000326714 + ENSR00000148786 + ENSR00000005553, with the strongest correlation (R=0.84, P=2.1e-6; Figure 1D). Of note, no improvement was observed after adding the two protein-coding genes (LCP1 and ADPGK) from a model reported previously (18) (Table S2). Although showing slightly lower performance than the previous protein-coding gene model (LCP1+ADPGK), our enhancer-based model, explaining 71% (R-squared, R=0.84) of irAE variance, demonstrated that eRNAs alone can effectively predict irAEs.

Three eRNAs effectively predict immunotherapy benefit

Similarly, we selected the top ten eRNAs with nominal significance (P<0.05) of positive correlation with ORRs (Table S3, ENSR00000187665 with the highest correlation was shown

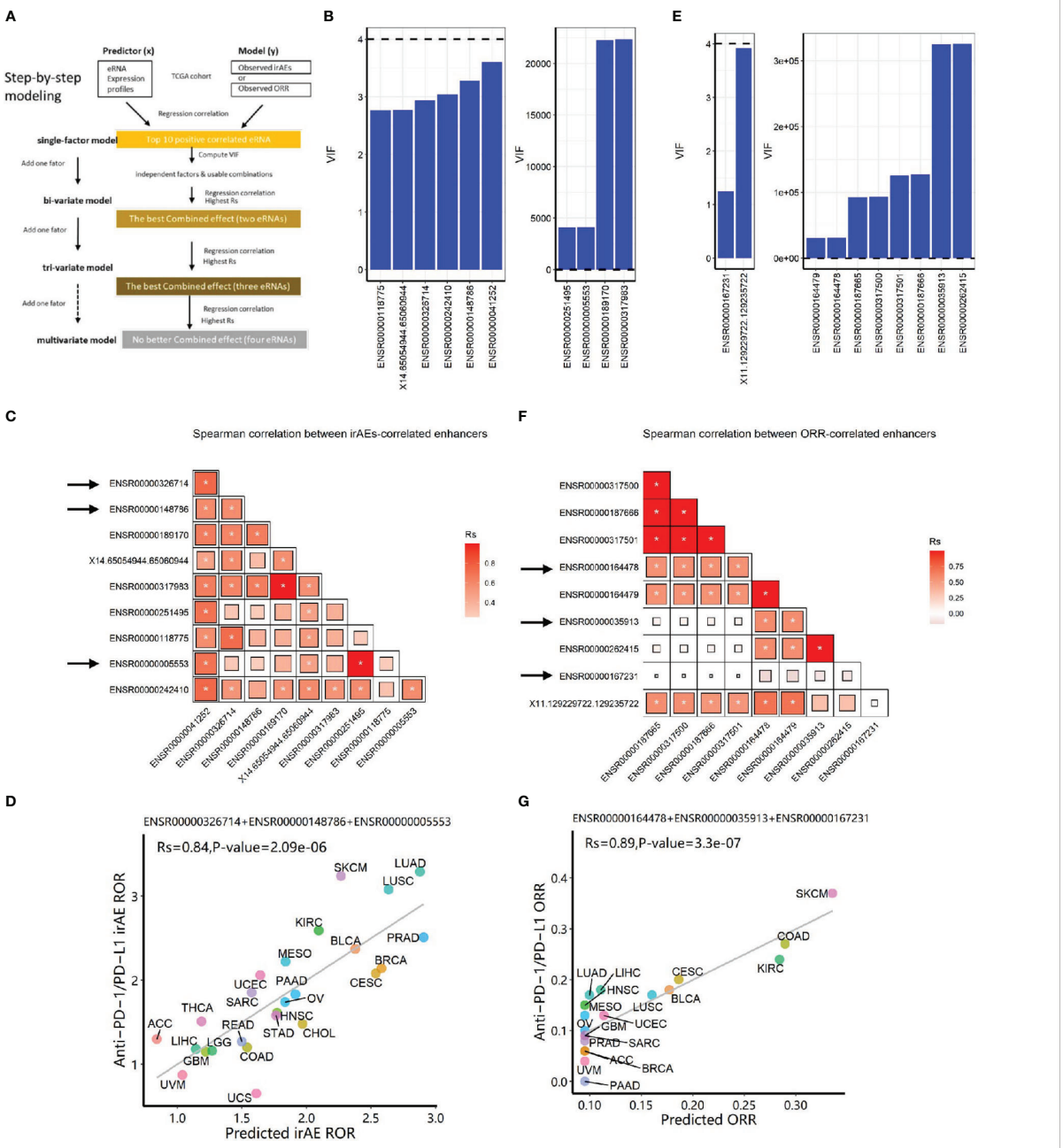

FIGURE 1
Construction of eRNA-based prediction models for irAE ROR (risk) and ORR (benefit) of immunotherapy. **(A)** The step-by-step workflow of this study. **(B)** Multicollinearity (VIF) analysis for top ten eRNA expression in predicting irAEs. Six eRNAs showed no multicollinearity, while 4 eRNAs showed strong multicollinearity. **(C)** Spearman correlation (Rs) between irAE-correlated eRNAs. The shade of the square indicates the Rs, and the size indicates significance (* indicates statistical significance $P < 0.05$). **(D)** Combined effects of the final trivariate model of predicting irAEs (R=0.84, *P*=2.1e-6). The model is 0.1912*ENSR00000005553+0.4097*ENSR00000326714+0.1953*ENSR00000148786+0.2942.
(E) Multicollinearity analysis for top ten eRNA expression in predicting ORR. Two eRNAs showed no multicollinearity, while 8 eRNAs showed strong multicollinearity. **(F)** Spearman correlation between ORR-correlated eRNAs. The shade of the square indicates the *Rs*, and the size indicates P-value (* indicates statistical significance *P*< 0.05). **(G)** Combined effects of the final model of predicting ORR (R=0.89, *P*=3.3e-7). The model is 0.0953 + 0.0649*ENSR00000164478+0.0032*ENSR00000035913+0.1687*ENSR00000167231. irAE, immune-related adverse events; ROR, reporting odds ratio; ORR, objective response rates; LUAD, lung adenocarcinoma; SKCM, skin cutaneous melanoma; LUSC, lung squamous cell carcinoma; KIRC, kidney renal clear cell carcinoma; PRAD, prostate adenocarcinoma; BLCA, bladder urothelial carcinoma; MESO, mesothelioma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; UCEC, uterine corpus endometrial carcinoma; SARC, sarcoma; ESCA, esophageal carcinoma; PAAD, pancreatic adenocarcinoma; OV, ovarian serous cystadenocarcinoma; HNSC, head and neck squamous cell carcinoma; STAD, stomach adenocarcinoma; THCA, thyroid carcinoma; CHOL, cholangiocarcinoma; ACC, adrenocortical carcinoma; READ, rectum adenocarcinoma; COAD, colon adenocarcinoma; LIHC, liver hepatocellular carcinoma; LGG, brain lower-grade glioma; GBM, glioblastoma multiforme; UVM, uveal melanoma; UCS, uterine carcinosarcoma.

in Figure S1C). After multicollinearity analysis (Figures 1E, F), two bivariate models achieved better predictive performance than single-eRNA models (one shown in Figure S1D; R=0.82, *P*=2.0e-5). Further adding model factors resulted in four equally-efficient optimal trivariate models (involving five eRNAs, Table S4) for ORR prediction were able to effectively predict the efficacy of anti–PD-1/PD-L1 treatments. One example, ENSR00000164478 + ENSR00000035913+ ENSR00000167231, was shown in Figure 1G (R=0.89, *P*=3.3e-7).

eRNA ENSR00000148786 may target TMEM43 to induce irAE during immunotherapy

Enhancers were assumed to affect irAEs or ORRs by activating target genes through long-range interactions. We downloaded enhancer-target interaction data (21) and obtained putative targets of our enhancers. Two eRNAs (ENSR00000262415 and ENSRO0000167231) were excluded from downstream analysis due to lack of any annotated target gene. eRNA-target networks showed that these enhancers independently regulated a specific groups of targets (Figure 2A for irAE and Figure 2B for ORR, note that ENSR00000164478 and ENSR00000164479 located to the same genomic region), indicating that each irAE-related enhancer was involved in different regulatory modules. Similarly, protein-protein interaction (PPI) analysis revealed that an independent network was controlled by each enhancer (Figures 2C, D). In these PPI networks, genes located in the center (such as BCL7B, TBL2, and NAP1L4) might be vital regulators of irAEs or ORRs. However, although both BCL7B and TBL2 were closely related to functions of the immune system, no connection between NAP1L4 and immune functions was reported.

Then, we examined associations between eRNA targets and irAE or ORR. First, we found that these eRNA-target genes were not among top irAE-related factors reported in the study by Jing et al. (18). We observed the best correlations between SPDYE7P and irAE (R=0.64, *P*=5.1e-4) and between PCYT1A and ORR (R=0.54, P=0.016). After multiple testing correction, only SPDYE7P (speedy/RINGO cell cycle regulator family member E7, pseudogene) and TMEM43 (transmembrane protein 43, also known as LUMA) showed correlation with irAE and with FDR<=0.1. Interestingly, TMEM43/LUMA (chr3:14,124,940-14,143,679), a putative target of irAE-associated ENSR00000148786 (chr3:13,346,900), is known to be able to modulate the innate immune pathways, which can probably induce irAE. Specifically, TMEM43 can form a protein complex with ENDOD1, TMEM33, and TMED1 to promote cGAS-STING signaling (22). It can also activate NF-kB signaling *via* interaction with CARD10 and its associated complex (23). The Spearman correlation between ENSR00000148786 and irAE was 0.66 (P=3.7e-4, Figure 3A) and the one between TMEM43/LUMA and irAE was similar (Rs=0.56, P=0.0045, Figure 3B).

More importantly, both PD-1 and PD-L1 are critical regulators of B cell functions, which subsequently affect functions of T cells and other immune cells (24, 25). Surprisingly, by using the eRic database (21), we found that ENSR00000148786 expression was the highest in a B-cell malignancy, DLBC (diffuse large B-cell lymphoma) (Figure 3C), in which ENSR00000148786 also showed the highest correlation with TMEM43/LUMA (Rs=0.59, FDR= 8.4e-4, Figure 3D). It is possible that the much lower levels ENSR00000148786 from tumor types other than DLBC were also originated mainly from tumor-infiltrated B cells in the microenvironment. Although we did not find direct correlations between other eRNA targets and irAE or ORR, those eRNA targets may exert their functions combinatorically.

Enhancer targets reveal metabolic and inflammatory genes involved in irAEs

Next, we downloaded gene sets from COSMIC (26) and oncoKB (27) and examined our eRNA targets in known oncogenic signaling pathways using cBioPortal (28, 29). We found that some eRNA targets were known cancer genes relevant to tumor immunity, including *MLXIPL, MPL, RAF1*, and *XPC*. *RAF1* was annotated as an oncogene and participated in the RTK-RAS signaling pathway (Figure 4A), and MLXIPL was involved in MYC signaling pathway (Figure 4B). A previous study (30) showed that RAF1 can activate MAPK1 and NF-κB pathways to regulate genes involved in inflammation. Therefore, RAF1 may enhance immunoreaction and subsequently cause irAEs *via* Natural Killer cell-mediated cytotoxicity, T cell receptor signaling pathway, and B cell receptor signaling pathway, based on functional annotations of RAF1 (Table S5).

Interestingly, we found that ENSR00000326714 targets were enriched in a large number of metabolic and biosynthesis processes (Figure 4C). This was reminiscent of some types of adverse events, such as diabetes (16), due to metabolic disturbances or metabolic disorders. Specifically, the core network of ENSR00000326714 targets consists of seven metabolic and inflammatory genes, namely, BAZ1B, BCL7B, TBL2, MLXIPL, NSUN, STX1A, and VPS37D. Among them, BAZ1B, BCL7B, TBL2 and MLXIPL are pleiotropic genes for lipids and inflammatory markers in the liver (31). Of note, MLXIPL encodes the carbohydrate-responsive element-binding protein (ChREBP), which mediates glucose homeostasis and liver lipid metabolism. ChREBP was also associated with up-regulation of several cytokines (TNF-α, IL-1β, and IL-6) in patients with type 2 diabetes mellitus, promoting the inflammatory responses and apoptosis of mesangial cells (32). STX1A encodes a member of the syntaxin superfamily, syntaxin 1A. It contributes to neural function in the central nervous system by regulating transmitter release (33). As a kind of target-SNAP receptor (t-SNAREs), it is involved in insulin exocytosis

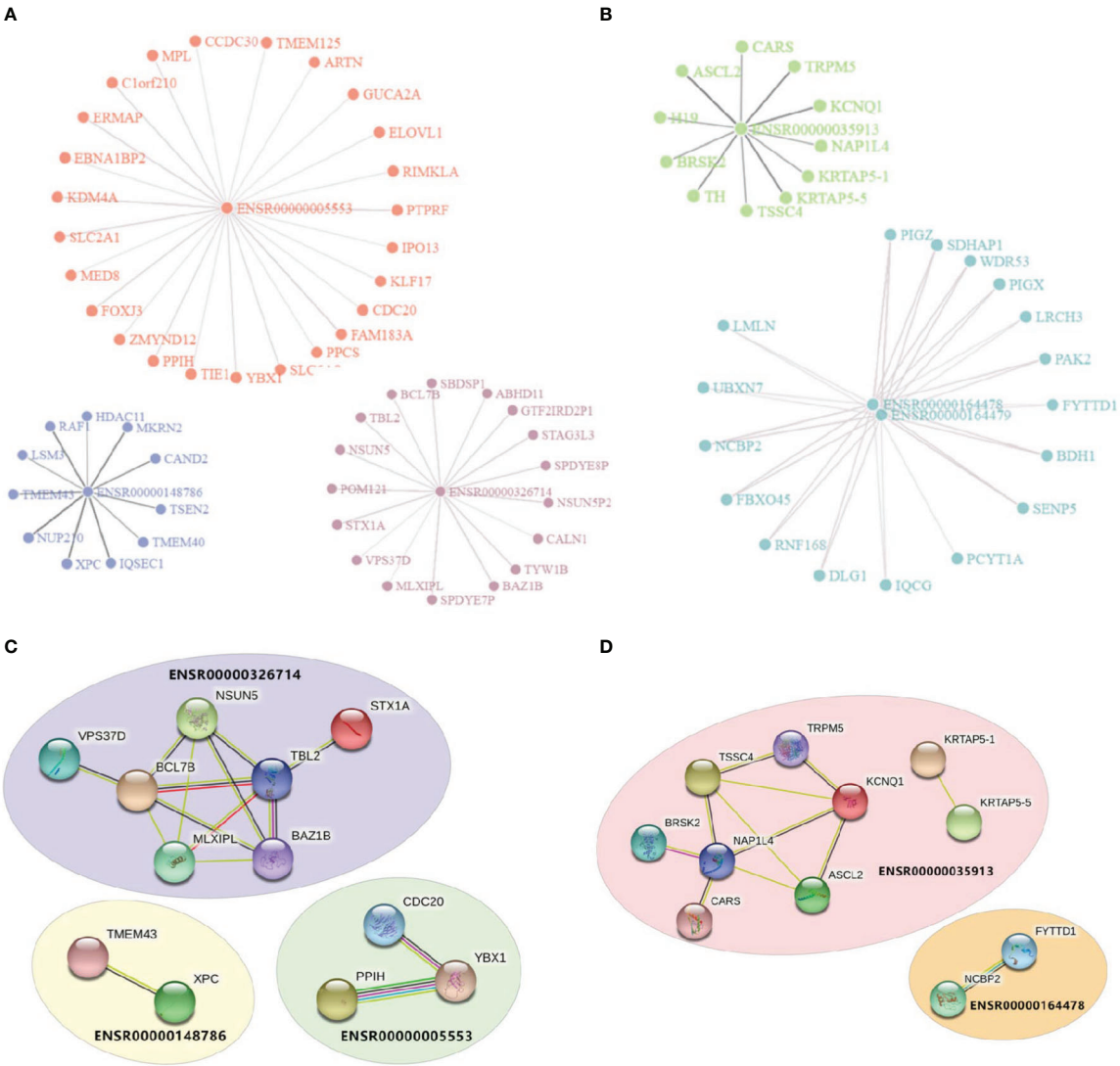

FIGURE 2
Visualization of enhancer-target interaction network and functional enrichment. **(A)** Target genes of irAE-related enhancers ENSR00000005553, ENSR00000326714, and ENSR00000148786. **(B)** Target genes of ORR-related enhancers ENSR00000164478, ENSR00000164478, and ENSR00000035913. **(C, D)** Protein-Protein Interaction (PPI) networks of target genes of enhancers in the prediction models of irAE **(C)** or ORR **(D)**.

(34). Severely reduced islet syntaxin 1A level was reported to contribute to insulin secretory deficiency (35). Given that diabetes and hepatitis account for ~30% of immune-related adverse events (16), we speculate that ENSR00000326714 probably plays a role in toxic effects in these patients.

ORR enhancers reveal immune activation genes for immunotherapy benefit

We also analyzed target genes of ORR-predictable eRNAs, which included three types of genes. PAK2, LMLN, DLG1, ASCL2, SENP5, IQCG, and BRSK2 are related to cell cycle, cell division, and differentiation. PIGZ, PIGX, PCYT1A, CARS, and BDH1 are metabolic genes; TRPM5, KCNQ1, and FYTTD1 are responsible for cellular transport and signal transduction. In particular, target genes of ORR-related ENSR00000164478 were enriched in glycosylphosphatidylinositol (GPI)-anchor biosynthesis (FDR=4.73×10^{-3}) (Figure 4D) and T-cell receptor signaling (FDR=3.78×10^{-2}), among other enriched pathways (Figure 4E).

Furthermore, PAK2 and DLG1 are involved in the T cell activation pathway (Table S5), which may explain their connection with ORR. P21 (RAC1) activated kinase 2 (PAK2) has been reported as a key signaling molecule in the differentiation of T cells. PAK2 is essential in T cell development and differentiation (36), indicating its potential

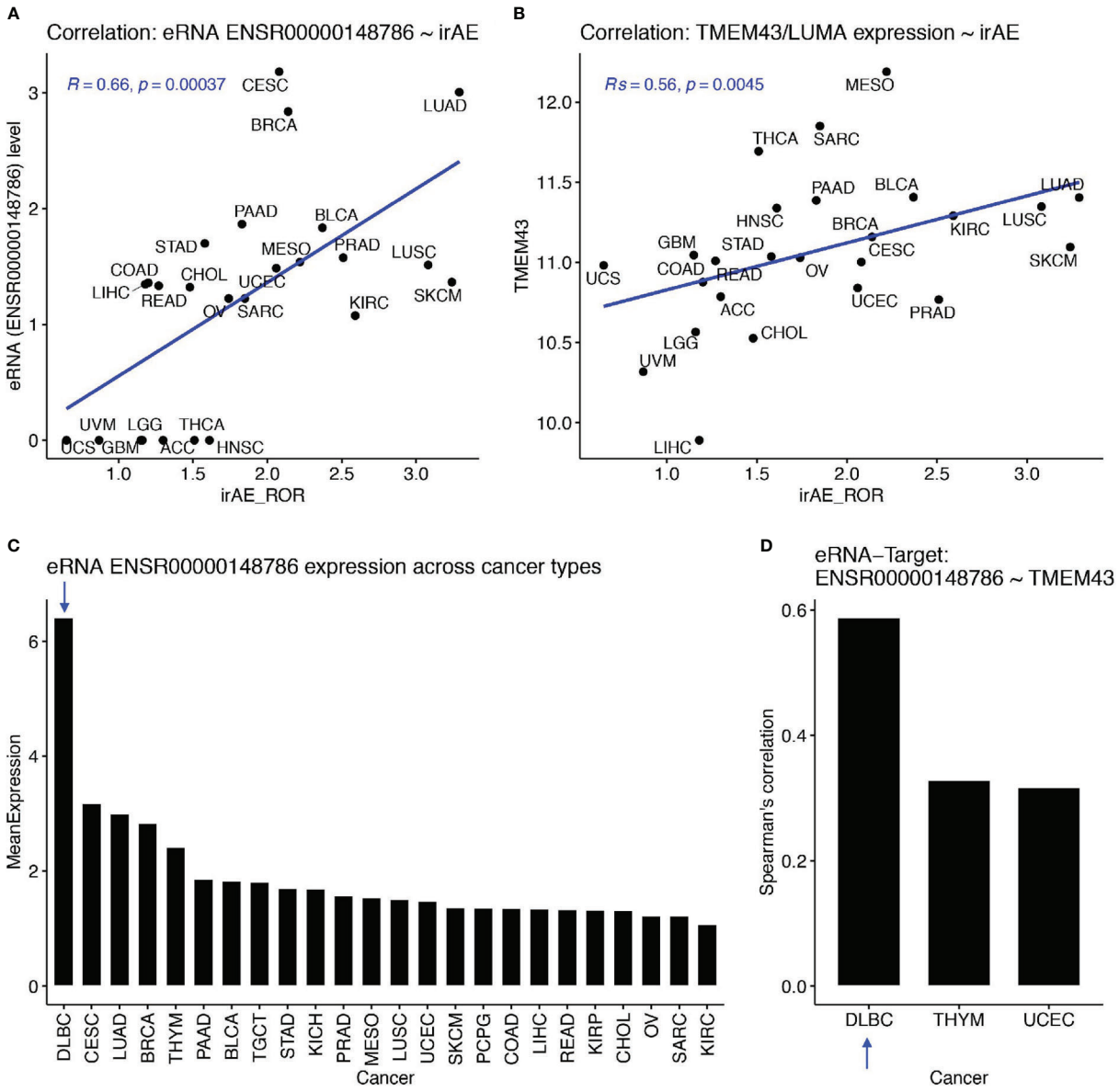

FIGURE 3
The ENSR00000148786 putative target TMEM43/LUMA potentially induced irAE from B cells. **(A, B)** Significant correlation between irAE and expression levels of eRNA ENSR00000148786 **(A)** or TMEM43/LUMA **(B, C)** Sorted mean expression levels of eRNA ENSR00000148786 across cancer types, with the arrow indicating the highest expression in DLBC (diffuse large B-cell lymphoma). **(D)** Significant eRNA-target correlation between ENSR00000148786 and TMEM43/LUMA was the highest in DLBC among the three significant cancer types.

function in T cell-initiated autoimmunity. DLG1 encodes a multi-domain scaffolding protein from the membrane-associated guanylate kinase family, which has been shown to regulate the antigen receptor signaling and cell polarity in lymphocytes, involved in activation and proliferation of T cells (37, 38). Our results provide more support for the T cells as the regulators in immune responses during immune checkpoint blockade therapy. Lastly, PIGZ encodes a protein that is previously identified as an immune-associated prognosis signature (39). However, knowledge of the relationship between PIGZ and the immune system is still poorly established. The association between PIGZ expression and immune benefits during anti-PD1/PDL1 immunotherapy needs further elucidation.

Discussions

In this work, we presented a preliminary evaluation of the different enhancer-target interactions associated with anti–PD-

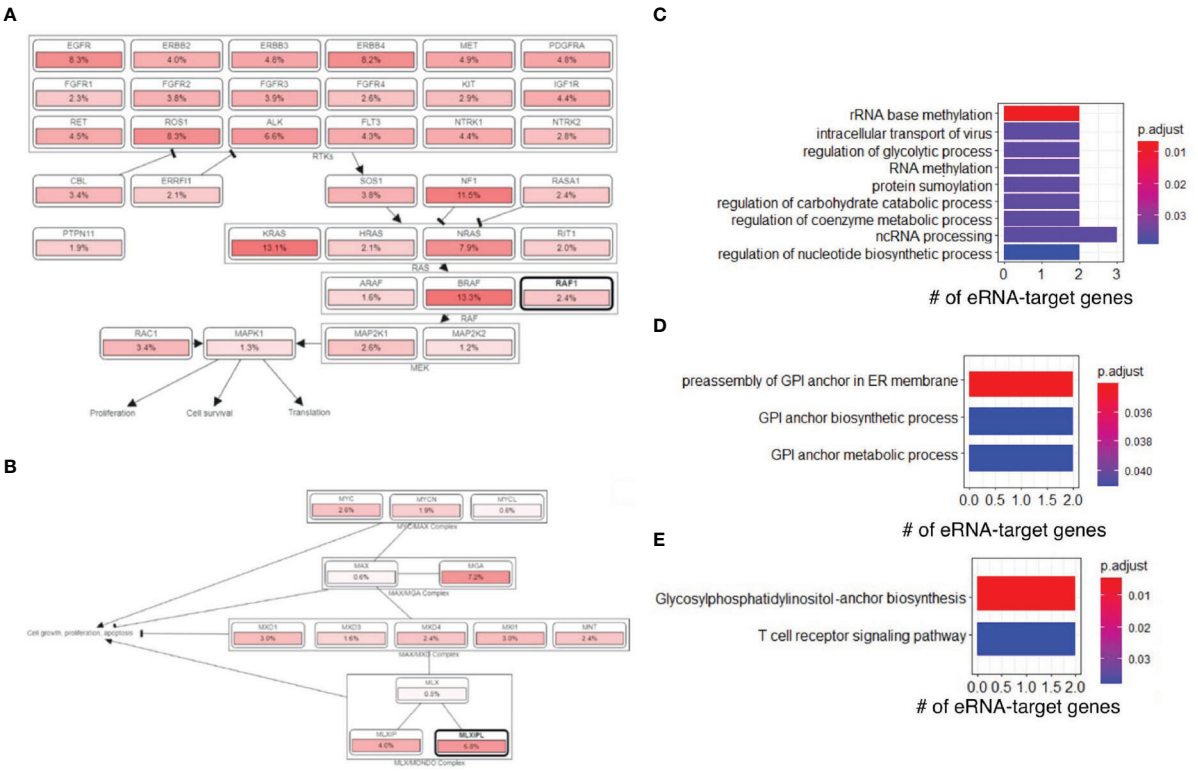

FIGURE 4
irAE-related genes were involved in oncogenic and other pathways. **(A)** The RAF1 and RTK-RAS signaling pathway. Pathway graphs were generated by using the cBioPortal website. **(B)** The MLXIPL and MYC signaling pathway. **(C)** GO enrichment of genes regulated by irAE-correlated enhancer ENSR00000326714. **(D)** GO enrichment of genes regulated by ORR-correlated enhancer ENSR00000164478. **(E)** KEGG pathway enrichment of genes regulated by ORR-correlated enhancer ENSR00000164478.

1/PD-L1 immunotherapy across tumor types, and successfully identified potential enhancer-based biomarkers of risk and beneficial responses. We suggest that, during immunotherapy, enhanced expression of inflammatory factors including TMEM43, BCL7B, TBL2, MLXIPL, STX1A, and RAF1 may lead to a higher risk of irAEs, while immune activation factors including PAK2 and DLG1, in addition to NAP1L4 whose function has not been related to the immune system currently, may improve anti-tumor immunity. Besides, we discovered many other cancer-related, metabolic, signaling or regulatory genes possess predictive potential, which warrants further investigation.

Several limitations remain for future work and our results need to be carefully interpreted. First, the majority of data are collected from previous individual studies (21), introducing inherent limitations of this work. Second, there are inevitable flaws of modeling as well, due to the low expression level of eRNA and small sample size. The overall quality of predictive models of ORR is inferior to those of irAEs, probably due to a smaller sample size as well as larger sparsity of ORR data. Moreover, we only considered eRNAs positively correlated with irAE or ORR. Although further adding eRNAs negatively correlated with irAE or ORR may not further improve model performance and probably cannot compete with mRNA-based models (18), identification of important negatively correlated eRNAs may contribute to the understanding of irAE or ORR mediated by enhancers. Third, another great eRNA and super-enhancer RNA (seRNA) study and its associated database (TCeA), with a much larger number of eRNAs and seRNAs, have been published recently (40), which we hope to integrate into our future work soon. Finally, since results in this project are mainly based on computational predictions and the support of existing literature, our findings need further experimental validation. A larger dataset is required to comprehensively model side effects or immune response as well.

Methods

Data collection

To quantify the risk of immune-related adverse events (irAEs), reporting odds ratio (ROR) was calculated as previously described (41). The anti-PD1/PD-L1 irAE ROR and

ORR values across different cancer types were collected from previous studies (10, 18). RNA-seq expression data (RSEM normalized counts, log2-transformed) across 25 TCGA cancers were downloaded from the UCSC Xena platform (http://xena.ucsc.edu/). Expression levels of selected genes were extracted for downstream analysis, and the average value was calculated for each TCGA cohort. We downloaded eRNA expression levels and enhancer-target associations for 7 045 enhancer RNAs in ~7,300 samples from the eRic database (21) (https://hanlab.uth.edu/eRic/). Mean eRNA expression (log2-transformed RPM values) were used. Similar to gene expression, we averaged the expression level of each eRNA for each cancer.

Prediction model construction

FDR control (requiring FDR<0.05) of *P*-values was too strict and resulted in exclusion of all eRNAs from building prediction models. There were mainly two reasons behind this problem. First, most eRNAs had low expression (compared to mRNAs) and many of their estimated expression levels were possibly affected by noise, which severely affected their *P*-values of correlation with irAE (or ORR). Second, the correlation between irAE (or ORR) and eRNAs were based on a small number of summarized data points (only one for each cancer type), further affecting the significance of *P*-values. Therefore, we decided to choose the top ten eRNAs ranked by *P*-values (<0.01) for downstream analysis.

First, the top ten eRNAs were selected based on correlation between eRNA and irAE or ORR. Before constructing bivariate models, the variance inflation factor (42) (VIF) of these ten eRNAs was calculated to evaluate the multicollinearity. Strong multicollinearity indicates redundancy of variables and should be avoided in the prediction models. Generally, we set the threshold of VIF value to 4 (a VIF value greater than 10 will be considered serious multicollinearity). The optimal prediction model was obtained by step-wise addition of model factors (eRNA) and evaluate the correlation between predicted and observed patient risk or benefits.

Bioinformatics tools

We used the protein-protein interaction (PPI) database STRING (43) (v11, https://string-db.org) to investigate selected eRNA target genes. Basic GO and KEGG term enrichment and visualization were conducted with the R package clusterProfiler (44) (v3.14.3). Extensive functional annotation of eRNA target genes were performed with DAVID (45) (v6.8) (https://david.ncifcrf.gov/). To verify cancer-related function for genes of interest, a credible set of 723 cancer genes was downloaded from the Cancer Gene Census (CGC) project of the COSMIC (26) repository (https://cancer.sanger.ac.uk/cosmic/). Another database oncoKB (27) (https://oncokb.org/), which has a list of 1,064 cancer genes, was added as a supplement to COSMIC CGC genes. Oncogenic signaling pathways were provided by the cBioPortal database (28) (http://www.cbioportal.org/). Statistical analysis and visualization were performed in R (v3.6.3) using packages ggplot2 (v3.3.2), networkD3 (v0.4). For novel candidates, we used three types of biological interpretation (Gene Oncology, Pathways, and Protein-Protein Interaction) to obtain biological knowledge.

Statistical methods

We employed an approach as described previously (10, 18) to evaluate the correlation between eRNAs and irAE RORs or ORRs. Linear-regression models for predicting irAE ROR or ORR across cancer types, was constructed by the R function lm, and the performance of the prediction was estimated based on Spearman rank correlation, using the R package psych (v2.0.12). To compare the goodness of fit between different models, a log-likelihood ratio test was performed using the R package lmtest (v0.9). We compute variance inflation factor (VIF) to assess multicollinearity using the vif function from the R package car (v3.0) to exclude combinations containing highly correlated factors.

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

YX and MG conceived and supervised the study. ZL, YX, and MG performed the analysis. MG drafted the manuscript with assistance from ZL. YX reviewed the manuscript. All authors approved the final manuscript. All authors contributed to the article and approved the submitted version.

Funding

The research has been supported by National Natural Science Foundation of China (NSFC) (Grant 31571350, U1611265, and 31871323) and Guangdong Basic and Applied Basic Research Foundation (2021A1515110972). The results shown here are in whole or part based upon data generated by the TCGA or CPTAC Research Network.

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/fonc.2022.1048127/full#supplementary-material

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