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REVIEW

CANCER INNOVATION

Advances in research on molecular markers in immune checkpoint inhibitor-associated myocarditis

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Abstract

Immune checkpoint inhibitors (ICIs) play a crucial role in the immunotherapy of malignant tumors, preventing immune evasion by tumor cells and activating autoimmune cells to eliminate the tumor. Despite their proven effectiveness in antitumor therapy, potential immune-related adverse effects must be recognized, particularly ICI-associated myocarditis (ICIAM). ICIAM is the most lethal form of organ immunotoxicity, with a significant impact on short-term mortality. However, ICIAM is predominantly asymptomatic or mildly nonspecific. It is difficult to diagnose, especially due to the lack of unique molecular markers. This article aims to provide a comprehensive overview of the progress made in identifying molecular markers for ICIAM.

KEYWORDS

immune checkpoint inhibitors, molecular marker, myocarditis, tumor immunotherapy

1 | INTRODUCTION

Immune checkpoint inhibitors (ICIs) have shown great advantages and potential in tumor therapy [1]. Two types of ICIs have been used clinically: programmed cell death protein 1 and ligand (PD-1/PD-L1) inhibitors and peripheral blood cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) inhibitors. PD-L1 is located on the surface of tumor cells and binds to PD-1 on T lymphocytes, thereby inhibiting the activity of cytotoxic T cells [2]. CTLA-4 is highly expressed on the surface of tumor-infiltrating regulatory T cells (Treg cells) and binds to B7 on the surface of antigen-presenting cells (APCs). This pathway transmits inhibitory signals to reduce the immune response of T cells [3]. Consequently, tumor cells can exhaust T cells and achieve immune escape [4]. ICIs are monoclonal antibodies that block these immune checkpoints and restore the ability of T cells to fight tumors [5].

ICIs can lead to enhanced immune responses, but this can also lead to immune-related adverse events (irAEs) [6], of which cardiac irAEs are the most lethal [7–9].

Abbreviations: APCs, antigen-presenting cells; Bcl-2L12, Bcl-2-like protein 12; CircRNAs, circular RNAs; CRS, cytokine release syndrome; CTLA-4, cytotoxic T lymphocyte-associated antigen-4; cTnT/I, cardiac troponin T/I; ECG, electrocardiogram; H-FBPs, heart type-fatty acid binding proteins; hs-cTnT/I, high-sensitivity cardiac troponin T/I; ICIAM, immune checkpoint inhibitor-associated myocarditis; ICIs, immune checkpoint inhibitors; irAEs, immune-related adverse events; miRNAs, microRNAs; mRNA, messenger RNA; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PD-1/PD-L1, protein 1 and ligand; sST2, soluble growth stimulation expressed gene 2 protein; Th2, helper T cell 2; Th17, type 17 helper T cells; Treg cells, regulatory T cells; α-MyHC, α-isoform of myosin heavy chain.

Jun Shao and Chuanbin Liu contributed equally to this study and shared the first authorship.

The incidence of cardiac irAEs is 1.3%, while ICIAM is the most common type, accounting for 50.8% of all cardiac irAEs and having the highest mortality rate. In addition, the incidence of cardiac irAEs may be underestimated in patients treated with ICIs [10]. Previous studies have shown that the incidence of ICIAM is only 0.27%–1.14%, but its clinical features lack specificity, and the fatality rate can be as high as 50% [4, 11, 12]. Thus, early detection and intervention are crucial for patient survival. There is an urgent need for effective tools for the early diagnosis of ICIAM.

2 | ICIAM

There was no significant evidence that different types of ICIs lead to differences in the pathological staging of ICIAM. Autopsies of ICIAM patients revealed a predominantly $CD3^+$ T cell infiltration in the myocardium, mainly composed of $CD8^+$ cytotoxic T cells and $CD4^+$ helper T cells, the former being more abundant than the latter [13–15]. However, there are differences in the incidence of ICIAM based on the different types of ICI. The incidence of ICIAM was 0.05%–0.38% with PD-1/PD-L1 inhibitors alone [13, 16] and 0.06%–1.08% with CTLA-4 inhibitors alone [17], the latter being slightly higher than the former. In addition, the incidence of ICIAM in patients receiving two or more ICIs was 2.4%, which was significantly higher than that of monotherapy [18, 19]. However, the true incidence of ICIAM might be underestimated due to the lack of specific clinical symptoms, potential overlap with other cardiovascular diseases, diagnostic challenges, and overall lack of awareness of the disease.

In a cohort study, 122 ICIAM patients developed early symptoms, such as chest pain, weakness, and panic, within an average of 30 days of their initial ICI exposure [20]. However, late cardiovascular events (>90 days) are less well characterized and are often associated with a higher risk of noninflammatory heart failure, progressive atherosclerosis, hypertension, and death [21]. Although the incidence of ICIAM is low, the risk of death after ICIAM is 38%–46% [18]. In fact, according to the recommendations of the European Society of Cardiology Oncocardiology Guidelines, myocarditis is considered a serious irAE and forms the basis for permanent discontinuation of immunotherapy [22, 23]. The occurrence of such adverse cardiovascular events necessitates discontinuation of treatment, thereby worsening the patients' prognosis, and only a very few cases can be considered for the reintroduction of immunotherapy [18, 23]. Therefore, early diagnosis of ICIAM is crucial for improving the long-term survival of patients.

3 | POSSIBLE MECHANISMS OF ICIAM

The mechanism by which ICI leads to irAEs in nontarget organs such as the heart remains unclear. Four main hypotheses have been proposed [1–6, 24]: (1) ICIs may directly bind to cell surface proteins, such as CTLA-4 expressed in normal tissues, leading to T cell infiltration and complement-mediated tissue damage. (2) Identifying T cells that recognize antigens expressed by tumor cells may potentially enter the circulation and subsequently identify the same tumor antigens or similar tissue antigens in healthy tissues. Inhibition of PD-1 or CTLA-4 by ICI therapy may facilitate this process. (3) There is evidence that immune checkpoint inhibition can increase the levels of circulating cytokines in affected tissues and promote the infiltration of inflammatory molecules into nontarget tissues. (4) The use of ICI may lead to an increase in autoantibodies against target organs or promote the formation of new autoantibodies. Although ICIAMs are known to disrupt cardiac immune homeostasis, other underlying mechanisms of ICI-induced cardiotoxicity may remain, some of which remain unclear.

4 | ICIAM FEATURES AND DIAGNOSIS

ICIAM is the main form of ICI-induced cardiotoxicity and has the following three key features: (1) Low morbidity and high mortality. The incidence of ICIAM ranges from 0.06% to 0.27%, with fatal myocarditis occurring in less than 0.17% of cases. Despite this, the mortality rate of ICIAM is still as high as 50%. In recent years, due to researchers' emphasis on cardiotoxicity, the incidence of ICIAM has been on the rise, and related reports have increased [10, 25, 26]. (2) ICIAM often manifests itself in the early stages of treatment. Typically, the median time to ICIAM is approximately 34 days (interquartile range: 21–75 days) after ICI initiation [19]. Of note, cardiotoxicity, including myocarditis, may occur at any time during receipt of an ICI, and in some cases, delayed cardiotoxicity may occur up to 90 days after discontinuation of the ICI [27]. (3) Certain patient groups with specific risk factors may be more susceptible to ICIAM. These risk factors include age above 75 years, underlying cardiac disease, previous autoimmune disease, combination chemotherapy involving anthracyclines [10, 28], and combination chimeric antigen receptor T-cell therapy [29].

The diagnosis of ICIAM requires a comprehensive evaluation including clinical presentation, electrocardiogram

(ECG), cardiac imaging, pathology, and blood biomarkers. However, both clinical presentation and ECG lack specificity [1, 19, 30]. Furthermore, cardiac imaging has limited sensitivity [19, 31]. The invasiveness and surgical risks associated with myocardial biopsy have limited its widespread use [32]. Given the rapid progression and poor prognosis of ICIAM, clinicians urgently need to identify highly sensitive and specific molecular markers for early diagnosis.

5 | ICIAM MOLECULAR MARKERS

5.1 | Specific antigens in tumors

There are certain highly homologous antigens or epitopes between cardiomyocytes and tumor cells. ICIs may induce allosteric recognition of tumor homologous antigens by T cells [13]. Johnson et al. [13] performed autopsies on two patients who received combined anti-CTLA-4 and anti-PD-1 therapy and developed ICIAM. The results demonstrated the presence of high-frequency T cell receptor sequences in the heart, skeletal muscle, and tumor infiltrates in both patients. Whole-transcriptome sequencing revealed increased expression of inflammatory T-cell factors in the myocardium and increased expression of muscle-specific antigens, such as junctional and troponin antigens, in tumors. Interestingly, both cases presented with myositis and myocarditis. One study reported that up to 38% of cases of ICI-associated myositis also included myocarditis [33]. The histology and immunophenotype of skeletal muscle were found to be similar to cardiac muscle [34]. These studies support the possibility of a shared antigen theory [13, 35]. However, clinical evidence for a link between shared tumors and cardiac antigens remains lacking. Determining which epitopes are recognized by these T cell receptors among the large number of potential antigens is a daunting task. Additionally, further studies are needed to elucidate the pathogenic antigens and molecular mechanisms of ICIAM. Collecting antibody titers and T cell frequency data for cardiac and tumor-associated antigens (e.g., desmin and troponin antigens) in patients with and without myocarditis will be crucial to understanding the type and range of antigens associated with ICIAM.

5.2 | Specific immune cells and antibodies

ICI leads to the disruption of cardiac immune homeostasis by affecting tissue-based tolerance mechanisms [36] and peripherally regulated tolerance mechanisms. Consequently, central tolerance cannot completely eliminate autoreactive cells [37]. Moreover, ICIs promote the production of autoantibodies against myocardial tissue, leading to ICIAM in patients [24].

5.2.1 | Cardiac troponin I or troponin antibodies

Cardiac troponin and myosin are contraction-regulating proteins present in cardiomyocytes. In the case of autoimmune myocarditis, these proteins can act as antigens, triggering the production of specific antibodies [38]. Numerous studies confirmed the importance of cardiac autoantibodies in the pathogenesis of myocarditis and their role in identifying patients with myocarditis [38–41]. In a study by Lucas et al. [42], mice genetically deficient in the PD-L1 gene (on an MRL background) exhibited spontaneous lethal myocarditis, with high titers of anticardiac myosin autoantibodies and troponin I autoantibodies detected. However, there is no conclusive evidence that these antibodies or antibody-mediated immune responses lead to myocarditis in patients receiving ICIs. However, these antibodies have the potential as biomarkers to identify patiens at risk for myocarditis [43].

5.2.2 | α-isoform of myosin heavy chain (α-MyHC)-specific T cells

The α-MyHC (which is encoded by the gene *Myh6*) is unique to the heart, expressed only in the myocardium, and has been identified as a major autoantigen in patients with idiopathic dilated cardiomyopathy [44]. Moreover, patients with ICIAM may also present with dilated heart disease. Lv et al. [45] demonstrated that thymic $CD4^+$ T cells lack tolerance to α-MyHC, making them susceptible to this severe disease. The limited presence of α-MyHC-specific $CD4^+$ T cells in the blood of healthy individuals suggests that the body lacks central T-cell tolerance to the protein [46]. In contrast, the number of α-MyHC-specific T cells in the peripheral blood of myocarditis patients was significantly higher. Grabie showed that α-MyHC-specific T cells play a central role in autoimmune myocarditis in certain populations with a genetic predisposition to autoimmunity [34].

5.2.3 | $CD4^+$ T cells with high expression of Bcl-2-like protein 12 (Bcl-2L12)

Bcl-2L12, a member of the Bcl-2 protein family, acts as an antiapoptotic protein that also inhibits p53 to promote

tumor cell survival [47, 48]. Studies have shown that it is involved in impaired immune tolerance [49–51]. Chen et al. [52] found that in end-stage heart failure, $CD4^+$ T cells isolated from myocarditis hearts showed high expression of Bcl-2L12, leading to abnormal helper T cell 2 (Th2) polarization in the heart. This abnormality enhanced interleukin (IL)-4 expression and disrupted the apoptotic machinery, ultimately leading to increased infiltration of cardiac-specific cytotoxic T cells into the myocardium.

5.3 | Cytokines

ICI causes an increase in circulating cytokines, and ICIAM is triggered when cytokines accumulate to a certain threshold in nontarget tissues such as myocardium [24]. Hang et al. [53] demonstrated that the expression of specific cytokines, including IL-1β, IL-4, IL-10, and interferon-γ (IFN-γ), was significantly upregulated in the blood of patients with fulminant myocarditis. However, with appropriate treatment, the levels of these cytokines gradually decrease to normal levels. Ji et al. [54] conducted a study on crab-eater monkeys treated with a combination of ipilimumab and nivolumab. Their research results showed that: (1) The activation and proliferation of T cells were closely related to the increase of cytokine levels such as IL-4, IL-6, IFN-γ, and tumor necrosis factor (TNF)-α in the blood. (2) Observed upregulation of multiple chemokine receptor genes in the CXCR3-CXCL9/CXCL10 and CCR5/CCL5 axes associated with T cell homing. Both the CXCR3-CXCL9/CXCL10 and CCR5/CCL5 axes have been implicated in the regulation of inflammatory responses and the promotion of downstream cytokine release [55–57]. These results suggest that specific cytokines may have potential as biomarkers for ICIAM.

5.3.1 | IL-6

IL-6 is a major driver of inflammation in cytokine release syndrome (CRS), leading to enhanced B-cell and T-cell activity and the release of acute-phase response proteins [58, 59]. Increased IL-6 levels may increase the risk of cardiovascular complications, including myocardial ischemia and atherosclerosis [60, 61]. Therefore, elevated IL-6 may lead to ICIAM. However, it may also be affected by factors such as tumor cell necrosis or nontarget organ inflammation. The specific threshold of IL-6 levels required to diagnose ICIAM remains to be studied.

5.3.2 | Soluble growth stimulation expressed gene 2 protein (sST2)

sST2 is a member of the IL-1 receptor. Previous studies have shown that the IL-133/ST2 pathway involved in T cell-mediated immune responses [62]. sST2 exhibits low biological variability and high stability, making it a reliable marker. Elevated sST2 levels are associated with myocardial mechanical stress or inflammatory responses [63, 64]. Li et al. [65] analysis of sST2 in ICIAM patients suggested that it has the potential to serve as a molecular marker for the diagnosis of ICIAM. They found that when $sST2 \geq 87.5$ ng/mL, the sensitivity and specificity of ICIAM prediction were 90% and 100%, respectively. Furthermore, the study of Wang et al. [66] study highlighted the superiority of sST2 over cardiac troponin I (cTnI) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) in diagnosing fulminant myocarditis. Plasma sST2 levels were positively correlated with cTnI and NT-proBNP and negatively correlated with cardiac systolic function. These findings further support the utility of sST2 as a diagnostic molecular marker for ICIAM, with higher levels indicating increased myocardial fibrosis and poor cardiac remodeling [67, 68]. Meanwhile, sST2 has a significant independent predictive value for the prognosis of ICIAM patients [69, 70].

5.4 | Myocardial injury markers

5.4.1 | Cardiac troponin T/I (cTnT/I)

Troponin is the most sensitive markers for detecting myocardial injury and is widely used in clinical practice [30]. Studies have shown that in cases of ICI-induced cardiotoxicity, troponin levels were elevated in 94% of patients and that both peak troponin levels and final troponin levels were associated with adverse outcomes. In patients with myocarditis, final cTnT levels ≥1.5 ng/mL are associated with a four-fold increased risk of adverse cardiovascular events [19].

5.4.2 | High-sensitivity cardiac troponin T/I (hs-cTnT/I)

Elevation of hs-cTnT/I is a specific indicator of cardiac injury and is characterized by re-expression of the cardiac isoform of troponin T/I in damaged and regenerating myocardium [71]. Petricciuol found that hs-cTnT/I ≥ 14 ng/L before medication could predict the occurrence of cardiotoxicity and adverse cardiovascular

events within 3 months of medication [72]. Consequently, the hs-cTnT/I can detect the sustained damage to trace amounts of myocardial tissue caused by ICI, thereby enabling early diagnosis of subclinical cardiac injury, especially in asymptomatic patients.

5.4.3 | NT-proBNP

NT-proBNP is crucial for the early diagnosis of ICIAM. A real-world study [25] involving 204 patients treated with ICIs showed that NT-proBNP levels were significantly elevated and periodically changed over time in patients experiencing adverse cardiovascular events, including post-ICI myocarditis. These findings suggest an NT-proBNP trend indicative of ICI-induced myocardial injury.

5.4.4 | Heart type-fatty acid-binding proteins

Fatty acid binding protein is a small cytoplasmic protein that is highly expressed in tissues with active fatty acid metabolism, such as heart and skeletal muscle. Yuan et al. [73] demonstrated that heart type-fatty acid binding proteins (H-FBPs) showed elevated levels at 3 months in ICI-treated patients with myocardial injury, whereas traditional molecular markers such as cTnI and NT-proBNP did not. This suggests that H-FABPs may serve as a more sensitive molecular marker for the detection of ICIAM.

5.5 | Noncoding RNA

5.5.1 | MicroRNAs (miRNAs)

miRNAs are noncoding RNA sequences that regulate posttranscriptional gene expression by targeting the 3′ untranslated regions of messenger RNA (mRNA) sequences [74]. Gene expression studies have shown that miRNAs are differentially expressed in heart disease [75]. These miRNAs remain stable in circulation and can be effectively amplified using sequence-specific amplification to increase the sensitivity and specificity of detection.

MiR-208a is the only cardiac-specific miRNA that is minimally affected by noncardiac tissue damage. According to the study of Wang et al. [76], elevated cardiac-specific miR-208a in plasma could serve as a promising biomarker for early detection of myocardial injury in humans. The study showed that miR-208a exhibited peak elevation before cTnI, suggesting its potential for early detection. Furthermore, miR-208a exhibited comparable sensitivity and specificity to cTnI, further highlighting its diagnostic significance.

Blanco-Domínguez et al. [77] found a significant increase in cardiac myosin-specific type 17 helper T cells in mice with autoimmune myocarditis. They identified a novel miRNA (mmu-miR-721) as a potential myocarditis marker by miRNA microarray analysis. Its human homolog hsa-miR-Chr8:96 is expected to be used in the molecular diagnosis of ICIAM.

Wang and Han [78] found specific miRNAs related to the heart, such as miR-1, miR-133a, miR-208a, miR-208b and miR-499, as well as immune status-related miRNAs, including miR-223-3p, miR-21, miR-146b, miR-155, miR-98, miR-93, miR-590-3p, miR-214 are related to myocarditis. These miRNAs play a role in promoting cardiac inflammation and may serve as reliable diagnostic molecular markers.

Most studies are investigating the utility of individual miRNAs as molecular markers. However, combining multiple miRNAs is expected to significantly improve diagnostic accuracy. In addition, larger studies are essential to define precisely the threshold for measuring cardiac-specific miRNAs in plasma to diagnose ICIAM.

5.5.2 | Circular RNAs (circRNAs)

circRNAs are a class of noncoding single-stranded RNAs with covalently closed continuous loops formed by back-splicing of pre-mRNAs. Due to the absence of 5′-3′ polarity and poly(A) tail, circRNA exhibits high stability, making it a potential new biomarker for disease diagnosis [79]. It has been demonstrated that circRNAs play a crucial role in the pathophysiology of cardiovascular diseases [80, 81].

Zhang et al. [82] found that has-circ-0071542 was significantly upregulated in children with fulminant myocarditis, which was subsequently named circACSL1. In the acute phase of myocarditis, the expression of circACSL1 increased significantly, but decreased in the recovery phase, indicating its correlation with myocarditis. Furthermore, the study observed that circACSL1 expression levels changed in line with the trends of hs-TnT and NT-proBNP, confirming that circACSL1 exacerbates myocardial inflammation and injury through the miR-8055/MAPK 14 pathway [83]. These findings suggest that circACSL1 has the potential to serve as a novel biomarker for the diagnosis of ICIAM.

6 | SUMMARY AND OUTLOOK

Although the incidence of ICIAM is relatively low, the application of ICIs shows good potential, and with the widespread use of ICIs, the number of ICIAM patients has gradually increased. ICIAM is characterized by rapid

progression, high mortality, and poor prognosis, requiring high clinical vigilance. Molecular markers associated with ICIAM play a crucial role in the early identification and diagnosis of the disease. However, most studies of ICIAM have been conducted on animal models and patients diagnosed with ICIAM. The limitations of ICIAM diagnosis and the lack of longitudinal data on the onset of ICIAM patients pose significant challenges to the study of ICIAM pathogenesis and molecular markers.

Currently, there is a lack of specific molecular markers for the diagnosis of ICIAM [84]. Urgently needed are molecular markers that combine specificity and sensitivity in the clinical setting. Additionally, studying the possible mechanisms and molecular markers of ICIAM can optimize the drug structure of ICIs, develop adjuvants to reduce ICIs-related cardiotoxicity, treat ICIAM and improve prognosis, such as TNF-α inhibitors [85], IL-6 inhibitors [29], and CTLA-4 agonists.

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Jun Shao: Investigation (equal); writing—original draft (equal). **Chuanbin Liu:** Investigation (equal); writing—original draft (equal). **Jing Wang:** Investigation (equal); writing—original draft (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

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ETHICS STATEMENT

Not applicable.

INFORMED CONSENT

Not applicable.

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