

From tumor immunotherapy to myocardial injury: A mechanistic discussion of immune checkpoint inhibitor-related myocarditis (Review)

LEIYANG DAI, YONG DUAN and QIUXIA XIONG

Department of Clinical Laboratory, The First Affiliated Hospital of Kunming Medical University, Kunming, Yunnan 650000, P.R. China

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Abstract. Immune checkpoint inhibitors (ICIs) have revolutionized cancer therapy, markedly improving the survival rate of numerous malignancies. However, the emergence of immune-related adverse events, particularly ICI-related myocarditis, poses substantial clinical challenges. Despite its relatively low incidence, ICI-related myocarditis demands urgent attention due to high mortality rates and treatment-related cardiovascular toxicity. The present review provides an in-depth analysis of the immunopathological mechanisms of ICI-related myocarditis, with a focus on the physiological role of immune checkpoints, the core mechanisms of immune tolerance failure following ICI blockade, the influence of genetics and the microenvironment, and the effects of myocardial electrophysiology and hormones. Attention is also given to the cellular and molecular mechanisms, particularly the roles of T cells and macrophages. The current review highlights the critical interactions between these factors in the initiation and progression of ICI-related myocarditis. In addition, clinical manifestations, diagnostic criteria incorporating advanced imaging and biomarker profiling, and differential diagnosis considerations are summarized, underscoring the necessity of multidisciplinary collaboration for timely intervention. Mechanistic elucidation of ICI-related myocarditis will enable preventive strategies, optimized early diagnostics and improved patient outcomes, and may ultimately reduce the clinical incidence of this disease.

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1. Introduction

As the core regulatory mechanism of immune homeostasis, immune checkpoints prevent autoimmune damage by regulating T-cell activity (1). However, in the tumor microenvironment, tumor cells evade immune surveillance by overexpressing PD-L1, which engages PD-1 on activated T cells, leading to functional suppression and T cell exhaustion (2); this discovery has driven the clinical translation of immune checkpoint inhibitors (ICIs). As monoclonal antibody drugs, ICIs reactivate T cell-mediated antitumor effects by blocking inhibitory signaling pathways (3). Since the approval of the first CTLA-4 inhibitor by the US Food and Drug Administration (FDA) in 2011, PD-1/PD-L1 inhibitors have expanded to the treatment of over a dozen solid tumors, including melanoma and non-small cell lung cancer, markedly improving patient survival rates (4). Notably, the 5-year survival rate for advanced melanoma increased from 15% with traditional therapies to 50% with the use of ipilimumab monotherapy or combination regimens involving PD-1 inhibitors such as nivolumab (5). However, alongside the therapeutic breakthroughs, immune-related adverse events (irAEs) have emerged as a new clinical challenge. Among these AEs, cardiovascular toxicity, particularly ICI-related myocarditis, has a high mortality rate of 46%, although it has an incidence of <2% (6). The use of combination therapy further increases the risk (3-5); therefore, the early diagnosis and management of ICI-related myocarditis have become critical issues in the field of cancer immunotherapy (7-9).

Notably, the heart is considered a specific target of immune attack (10); however, the underlying reasons for the heightened susceptibility in specific patient populations and the pathological mechanisms driving ICI-related myocarditis remain incompletely elucidated. The present review aimed to explore the immunopathological mechanisms of ICI-related myocarditis. By summarizing the existing literature, the review aimed to identify critical knowledge gaps in

Correspondence to: Professor Qiuxia Xiong or Professor Yong Duan, Department of Clinical Laboratory, The First Affiliated Hospital of Kunming Medical University, 295 Xichang Road, Kunming, Yunnan 650000, P.R. China
E-mail: xiongqiuxia1@kmmu.edu.cn
E-mail: duanyong7@139.com

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current research and propose future directions, including the development of novel therapeutic strategies and molecular targets. Ultimately, this work aims to provide clinicians with actionable insights to optimize the safety and efficacy of immunotherapy while advancing prognostic outcomes for ICI-related myocarditis.

2. Epidemiological features of ICI-related myocarditis

The development of ICI-related myocarditis is closely associated with immune hyperactivation and therapeutic mechanisms, with combination therapies posing a particularly elevated risk. Epidemiological data indicate that monotherapy with ICIs results in myocarditis incidence rates of 0.5, 2.4 and 3.3% for anti-PD-1, anti-PD-L1 and anti-CTLA-4 inhibitors, respectively. However, combining PD-1 and CTLA-4 inhibitors increases the incidence to 2.4% (11), with mortality rising sharply from 36% (monotherapy) to 67% (7,12). Chinese cohort studies report a domestic ICI-related myocarditis incidence of 1.05-1.06%, consistent with global epidemiological trends (13,14). Identified high-risk factors include diabetes mellitus, heart failure, a history of acute coronary syndrome and advanced age (>80 years) (11,15). Although the overall incidence remains <2%, the high fatality rate renders ICI-related myocarditis a critical clinical challenge in cancer immunotherapy.

ICI-related myocarditis typically occurs within weeks of initiation, with a median onset of 34 days (IQR: 21-75 days), with timing and risk varying by cancer type (7,11). Patients with melanoma, especially those receiving anti-PD-1/CTLA-4 combination therapy, predominantly develop early-onset myocarditis (≤ 2 weeks) (11). Conversely, patients with thymic epithelial tumors (TETs) more frequently experience delayed-onset myocarditis (≥ 6 weeks), likely due to thymic dysfunction and impaired central immune tolerance predisposing to T cell-mediated autoimmunity (16). Patients with TETs also demonstrate a higher incidence of severe irAEs, contributing to their high myocarditis risk (17). Beyond tumor-specific factors, host characteristics also modulate risk, including advanced age (>64 years), elevated body mass index (>28 kg/m²) and prior cardiovascular medication use (15,18). These findings underscore the need for tailored surveillance strategies integrating both patient-specific and cancer-specific factors to enable early detection and timely intervention.

3. Clinical manifestations, diagnosis and differential diagnosis of ICI-related myocarditis

Clinical manifestations. The diagnosis of ICI-related myocarditis requires the integration of clinical manifestations, laboratory markers, electrocardiogram findings and imaging features. Patients typically present with chest pain, dyspnea, palpitations and fatigue. In severe cases, the condition may progress to heart failure, cardiogenic shock or cardiac arrest (19). Physical examination may reveal hypotension, tachyarrhythmias, jugular vein distension or other signs (cold, clammy skin, prolonged capillary refill and oliguria) of hemodynamic compromise (9,19,20) (Fig. 1).

Diagnosis

Laboratory and imaging profiles in ICI-related myocarditis. The laboratory profile of ICI-related myocarditis is typically characterized by elevated myocardial injury and cardiac stress biomarkers. Multiple studies have demonstrated abnormal elevation of cardiac troponin (cTn)T and cTnI in 46-94% of cases (21,22). This also discovered a critical threshold of cTnT ≥ 32 times the upper reference limit, which was significantly associated with adverse myocardial toxicity events (high-risk cohort), whereas levels below this threshold indicated a better prognosis (low-risk group). However, it was revealed that some patients may present without cTnI or creatine kinase (CK) elevation (22). The prevalence of elevated natriuretic peptides [B-type natriuretic peptide (BNP) and N-terminal pro-BNP (NT-proBNP)] exhibits interstudy variability: A large-scale study reported NT-proBNP elevation in 77.5% (n=185) of cases (23), contrasting with 100% positivity in a 14-patient cohort (22), suggesting that sensitivity may be sample size-dependent. While myoglobin, CK-MB, aspartate aminotransferase and lactate dehydrogenase often show nonspecific elevation (24,25), cTns demonstrate superior specificity over natriuretic peptides, which may be confounded by non-inflammatory left ventricular dysfunction or acute cardiac stress. This may lead to increased wall tension and neurohormonal activation, elevating BNP/NT-proBNP levels even in the absence of myocardial inflammation (23,26). Although individual biomarkers lack diagnostic specificity, their combined interpretation provides critical value in the evaluation of ICI-related myocarditis.

Electrocardiogram (ECG) abnormalities are essential diagnostic elements, detecting atrial fibrillation, ventricular arrhythmias and conduction disturbances. Inflammatory involvement of the cardiac conduction system may manifest as intraventricular conduction delay, PR prolongation or complete heart block (27). Cardiac magnetic resonance (CMR) notably enhances diagnostic accuracy through structural and functional assessment, with particular sensitivity in quantifying myocardial involvement (28). Prospective studies have validated the T1 mapping technology of CMR as achieving 92% sensitivity for myocardial edema detection via extracellular volume and T1 value quantification, enabling early identification of myocarditis (28,29).

Existing diagnostic criteria and procedures. Diagnostic confirmation of ICI-related myocarditis requires multimodal integration of clinical presentation, biomarker profiles and imaging findings. Current international consensus advocates the Bonaca diagnostic framework comprising four pillars (30): i) Characteristic symptoms (including chest pain and heart failure); ii) ECG abnormalities (ST-T changes and conduction blocks); iii) elevated myocardial injury markers (cTnT ≥ 14 ng/l or CK-MB ≥ 5 μ g/l); iv) CMR hallmarks (T2-weighted edema and late gadolinium enhancement). Salem *et al* (31) refined this protocol through a multidisciplinary team (MDT) model: Primary screening via ECG with high-sensitivity cTn, followed by CMR evaluation for suspected cases, culminating in MDT interpretation. While endomyocardial biopsy remains the diagnostic gold standard, its invasive nature and sampling variability restrict application to diagnostically ambiguous cases (20).

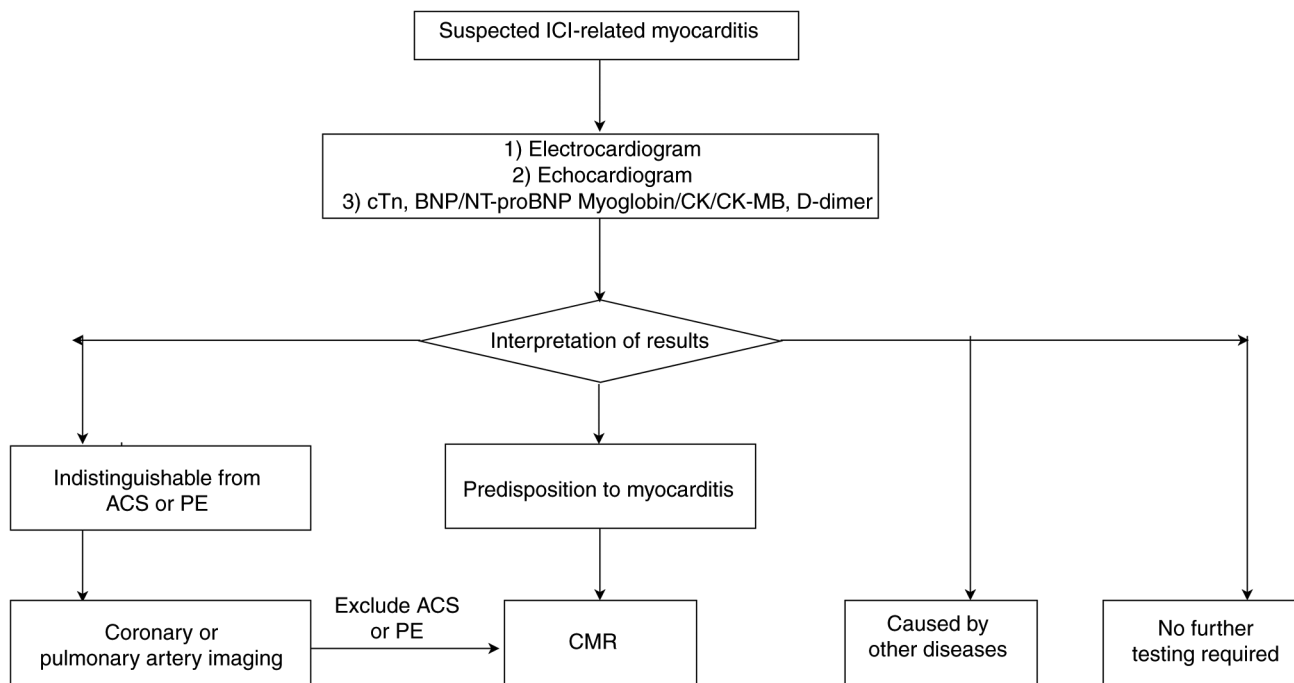


Figure 1. Examination and diagnostic process for suspected ICI-related myocarditis. ACS, acute coronary syndrome; BNP, B-type natriuretic peptide; CK, creatine kinase; CMR, cardiac magnetic resonance; cTn, cardiac troponin; ICI, immune checkpoint inhibitor; NT-proBNP, N-terminal pro-BNP; PE, pulmonary embolism.

Differential diagnosis

Core challenges in differential diagnosis. In clinical practice, establishing differential diagnosis for ICI-related myocarditis presents multifaceted challenges. First, the clinical manifestations demonstrate marked heterogeneity and nonspecificity, ranging from asymptomatic troponin elevation to fulminant heart failure or life-threatening arrhythmia. These presentations show substantial overlap with viral myocarditis and ischemic myocardial injury, while requiring differentiation from irAEs, particularly neuromuscular complications such as myositis and myasthenia gravis (32). Second, the coexistence of preexisting cardiovascular comorbidities and treatment-related complications in patients with cancer adds to the diagnostic complexity (33).

Laboratory assessments are confounded by limited specificity of conventional biomarkers (such as cTnT and CK), since nonspecific factors including skeletal muscle injury and systemic infections may alter their values. Furthermore, imaging modalities currently lack sufficient discriminatory power to reliably distinguish ICI-related myocarditis from other myocardial pathologies (34,35). Collectively, these diagnostic ambiguities underscore the critical need for developing novel biomarker panels and advanced imaging techniques to improve diagnostic precision.

Need for novel diagnostic tools and technologies. Given the limitations of current diagnostic methodologies, emerging technologies have expanded possibilities to address these challenges. Liquid biopsy demonstrates distinct advantages in early detection and disease stratification through the capture of circulating biomarkers, including circulating tumor cells and exosomes. These circulating components exhibit tissue specificity while dynamically reflecting alterations in the cardiac immune microenvironment, thereby providing

molecular evidence for differential diagnosis and therapeutic monitoring (36).

In artificial intelligence applications, machine learning-based risk prediction models integrating clinical characteristics, laboratory parameters and imaging biomarkers can effectively identify populations at high risk of adverse cardiac events. These models demonstrate superior predictive performance compared with conventional scoring systems, offering clinicians a robust foundation for early intervention strategies (37).

The synergistic application of multi-omics technologies (including genomics, proteomics and metabolomics) has further advanced the diagnostic paradigm for ICI-related myocarditis. Single-cell sequencing of epicardial adipose tissue has revealed that a CD8⁺ T cell to regulatory T cell (Treg) ratio exceeding 2.5 serves as an early indicator of severe myocarditis, enabling precision-guided immunosuppressive therapy (38).

4. Mechanism

Physiological functions of immune checkpoints and myocardial protection

PD-1/PD-L1 signaling pathway. Immune checkpoints serve key roles in maintaining immune homeostasis and regulating immune responses. Key immunosuppressive receptors, including CTLA-4, PD-1/PD-L1 and LAG-3, prevent immune system overactivation by modulating T-cell activation thresholds (39). The PD-1/PD-L1 axis exerts immunoregulatory functions through ligand-receptor interactions. As a CD28 superfamily member, PD-1 is ubiquitously expressed on activated T cells, B lymphocytes, monocytes, natural killer (NK) cells and dendritic cells (DCs). Upon PD-L1 engagement,

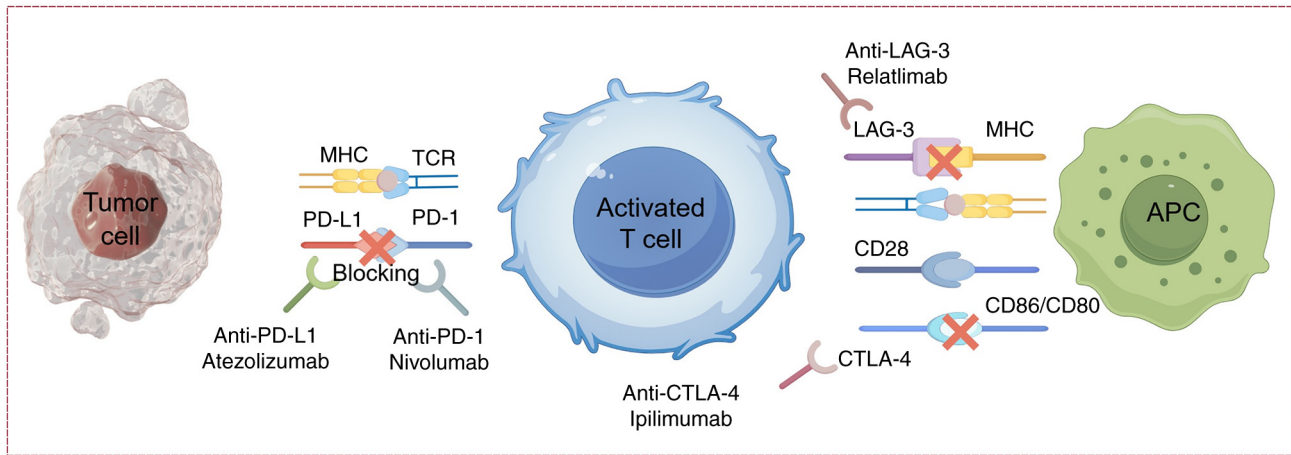


Figure 2. Physiological functions of immune checkpoints and myocardial protection. Physiological functions of immune checkpoints and myocardial protection. PD-1/PD-L1 signaling pathway, immunoregulatory function of CTLA-4 and the co-inhibitory role of LAG-3 are shown.

PD-1 recruits phosphatases SHP-1/2 to inhibit T-cell receptor (TCR) signaling, thereby maintaining peripheral immune tolerance (40). Constitutive PD-L1 expression on cardiomyocytes and endothelial cells provides local protection against autoimmune damage by simultaneously suppressing regional T-cell responses (41).

ICIs restore T cell-mediated antitumor activity through blockade of these inhibitory signals, potentially disrupting immune equilibrium. Anti-PD-1/PD-L1 monoclonal antibodies competitively inhibit PD-1/PD-L1 interactions, reversing T-cell functional suppression and activating downstream signaling pathways (such as PI3K/AKT and RAS/MAPK) to amplify immune responses (42,43). This mechanistic foundation has enabled PD-1 inhibitors, such as pembrolizumab, to achieve therapeutic breakthroughs across multiple solid malignancies, including tumors with specific genomic profiles (such as non-small cell lung cancer or black chromocoele) (44).

However, immune checkpoint blockade may reduce T-cell activation thresholds, permitting aberrant activation of autoreactive T cells that mount immune attacks against cardiac tissue, a central pathogenic mechanism underlying ICI-related myocarditis (45). The dual nature of therapeutic benefits and risks emphasizes the critical need for deeper understanding of immune checkpoint physiology and pathology (Fig. 2).

Immunoregulatory function of CTLA-4. CTLA-4, as a key immune checkpoint molecule, serves a critical role early in T-cell activation by competitively binding to CD80/CD86 molecules on the surface of antigen-presenting cells (APCs), antagonizing the CD28 co-stimulatory signal, and thus inhibiting T-cell proliferation and activation to maintain immune homeostasis (46,47) (Fig. 2).

The cardioprotective role of CTLA-4 is particularly notable: CTLA-4-deficient murine models develop lethal myocarditis and pancreatitis, demonstrating its essential function in cardiac autoimmunity (48). A previous investigative study from Harbin Medical University revealed that CTLA-4 m2a antibodies exacerbate myocardial damage in experimental autoimmune myocarditis through CCL5⁺ neutrophil subset infiltration, establishing a theoretical framework for preventing/treating CTLA-4-associated myocarditis (49). Concurrently, anti-CTLA-4 antibodies activate T helper

(Th)17 cells and aggravate pressure overload-induced heart failure, elucidating the immunological pathway through which CTLA-4 signaling dysregulation precipitates cardiac injury (50). In addition, CTLA-4 has a central role in Treg-mediated immune suppression by regulating cytokine secretion (such as by inhibiting the production of IFN- γ), T-cell differentiation and proliferation, and contact-dependent signaling between cells, thereby balancing the intensity of the adaptive immune response (51).

In oncological therapeutics, anti-CTLA-4 antibodies enhance T-cell activation via ligand binding blockade, thereby exerting antitumor effects (52). However, CTLA-4 inhibitors may induce cardiotoxicity, including *de novo* onset or exacerbation of heart failure. These findings underscore the necessity for systematic evaluation of the dynamic equilibrium between immune activation and cardiac function during treatment, mandating rigorous assessment of potential cardiovascular risks.

Co-inhibitory role of LAG-3. LAG-3, a type I transmembrane protein predominantly expressed on Tregs and exhausted T cells, exerts immunosuppressive effects on the tumor microenvironment through binding to MHC class II molecules on APCs, thereby inhibiting T-cell activation and proliferation (53). Mechanistically, LAG-3 demonstrates synergistic inhibitory effects with PD-1 in CD8⁺ T cells. Their co-expression potentiates T-cell exhaustion and suppresses IFN- γ -mediated antitumor immunity (54). This functional interplay has been substantiated in murine models of melanoma, where PD-1/LAG-3 double-deficient CD8⁺ T cells have been reported to exhibit enhanced tumor clearance and survival, while dual-knockout mice developed spontaneous T cell-mediated myocarditis, confirming their joint role in maintaining immune homeostasis and restraining autoimmune responses (Fig. 2) (55).

The clinical translation of this synergistic mechanism has yielded notable breakthroughs. Combination therapy employing anti-LAG-3 (such as relatlimab) and anti-PD-1 (for example, nivolumab) antibodies enhances T cell-mediated tumor cytotoxicity and promotes effector cytokine secretion, including TNF- α , IFN- γ and IL-2 (56). The landmark RELATIVITY-047 phase III trial demonstrated

superior clinical outcomes in patients with advanced melanoma receiving combination therapy vs. monotherapy, leading to its US FDA approval in 2022 (marketed as Opdualag) (57). This therapeutic milestone underscores the pivotal role of co-inhibitory receptor networks in advancing cancer immunotherapy paradigms.

Pathophysiological mechanisms of ICI-related myocarditis. To maintain immune homeostasis, the immune system must accurately distinguish between self and non-self antigens during the process of eliminating pathogens and malignant cells. This immunological balance is essential for mounting effective immune responses while simultaneously preventing autoimmunity. To achieve this, the host relies on two complementary tolerance mechanisms—central tolerance and peripheral tolerance. Disruption of these mechanisms, particularly through immune checkpoint inhibition, may lead to loss of self-tolerance and the development of immune-related adverse events, such as immune checkpoint inhibitor-related myocarditis (58).

Central tolerance primarily involves thymic negative selection, where self-reactive T lymphocytes are eliminated during development. Peripheral tolerance encompasses multifaceted regulatory processes: i) Functional suppression by Tregs and ii) dynamic modulation of T-cell activation thresholds through immune checkpoint signaling. These mechanisms collectively establish immunological safeguards, including the maintenance of immune privileged sites that protect vital organs from autoimmune attack.

The synergistic operation of central and peripheral tolerance systems enables accurate identification of pathogenic threats while preserving tissue integrity. Disruption of this equilibrium underlies the pathogenesis of ICI-related myocarditis, where checkpoint inhibition may override protective mechanisms, leading to aberrant immune targeting of cardiac tissue (59).

Destruction of immune tolerance mechanisms

Defects in central tolerance. Central tolerance establishment relies on a biphasic thymic selection process encompassing positive and negative selection (60). During positive selection, immature T-cell survival is determined by moderate-affinity interactions between TCRs and self-peptide-MHC complexes presented by thymic epithelial cells. T cells exhibiting optimal affinity thresholds differentiate into CD4⁺ helper or CD8⁺ cytotoxic T-cell lineages, whereas those with insufficient affinity undergo apoptosis due to failed survival signaling (61). Negative selection subsequently eliminates high-affinity autoreactive T cells through self-antigen presentation by medullary thymic epithelial cells (mTECs) and DCs (62). This dual filtration mechanism ensures exclusive export of foreign antigen-responsive mature T cells to secondary lymphoid organs, thereby maintaining immunological homeostasis. However, this system demonstrates critical deficiencies in purging tissue-specific antigens, particularly evident in cardiac autoimmunity.

The inefficacy of negative selection represents a key limitation in central tolerance. Thymic deletion fails to eliminate T cells targeting myosin heavy chain- α (MYHCA), a cardiac-specific protein encoded by the MYH6 gene and

predominantly expressed in the atrioventricular myocardium (63,64). This defect arises from the absence of MYH6 expression in the thymus. While mTECs mediate negative selection by regulating ectopic tissue antigen expression via the autoimmune regulator (63), impaired MYH6 transcription in mTECs allows MYHCA-reactive T cells to evade thymic deletion during development (65). These T cells therefore escape to peripheral tissues, where they may be activated under specific conditions, potentially triggering an autoimmune response. As a result, central tolerance is not entirely effective and relies on other mechanisms, such as peripheral tolerance, to compensate for its deficiencies, thus maintaining immune system homeostasis and preventing the onset of autoimmune diseases (Fig. 3A).

Disruption of peripheral tolerance. Peripheral tolerance maintains immune homeostasis by regulating the function of mature T cells. Its core mechanisms include the immunosuppressive function of Tregs and immune checkpoint signaling regulation. Tregs, as a subset of CD4⁺ T cells (accounting for 5–7%) (66), suppress the function of effector T cells through various mechanisms, including: Sequestration of the pro-survival signal molecule IL-2, secretion of anti-inflammatory cytokines such as TGF β , IL-10 and IL-35, direct lysis of APCs and effector T cells, mediating trogocytosis and inducing the generation of other immunosuppressive T cell subsets within the local microenvironment (67). Additionally, insufficient co-stimulatory signals during T-cell activation (such as low expression of CD80/CD86 on APCs) can lead to T-cell anergy or apoptosis, which is further suppressed by Tregs (68).

The immune checkpoint mechanism in peripheral tolerance mainly involves co-inhibitory receptors on the surface of T cells, such as CTLA-4, PD-1/PD-L1 and LAG-3 (69). CTLA-4 inhibits co-stimulatory signals by competitively binding to CD80/CD86, serving a negative regulatory role in the early stages of T-cell activation (4). PD-1 antagonizes the TCR/CD28 signaling pathway by recruiting tyrosine phosphatases, inhibiting the activation of effector T cells (70,71). In addition, CTLA-4 and PD-1 can enhance the immunosuppressive function of Tregs (72). LAG-3 interferes with the interaction between T cells and APCs by binding to MHC class II molecules (73). These molecules together constitute a dynamic regulatory network of peripheral tolerance.

The core mechanism by which ICIs induce cardiac autoimmunity lies in the dual disruption of peripheral tolerance. On one hand, ICIs block the CTLA-4 or PD-1/PD-L1 signaling axis, thereby relieving physiological suppression of effector T cells. This leads to their overactivation and subsequent attack on myocardial tissue (74). Experimental evidence has demonstrated that in PD-L1-deficient murine models of myocarditis, the inflammatory response is exacerbated, confirming the direct protective role of PD-1/PD-L1 in maintaining cardiac immune homeostasis (75). On the other hand, ICIs impair Treg function. For example, CTLA-4 deficiency induces fatal lymphoproliferative disorders, and anti-CTLA-4 therapy not only diminishes Treg-mediated suppression but also dysregulates PD-1 signaling, resulting in compounded tolerance defects (76). Additionally, ICI therapy disrupts endogenous cardiac protective mechanisms (such as myocardial PD-L1 expression), which potentiates CD8⁺ T cell

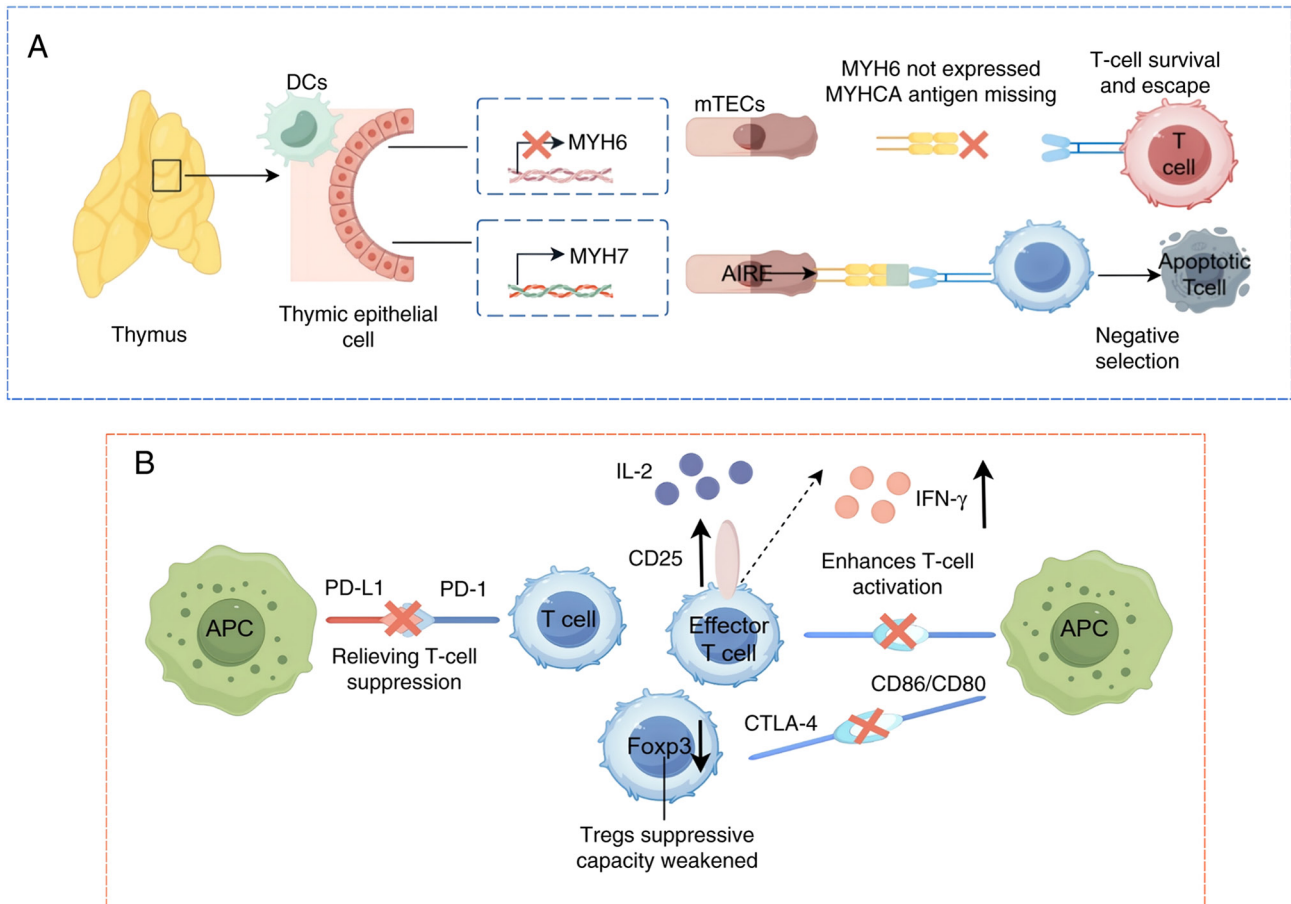


Figure 3. Mechanism of immune system tolerance imbalance in ICI-related myocarditis. (A) Defects in central tolerance. (B) Disruption of peripheral tolerance. APC, antigen-presenting cell; DCs, dendritic cells; ICI, immune checkpoint inhibitor; mTECs, medullary thymic epithelial cells; MYH, myosin heavy chain; Treg, regulatory T cell.

cytotoxicity against cardiac tissue (77). Concurrently, LAG-3 inhibition exacerbates inflammatory cardiomyopathy (78). The systemic collapse of peripheral tolerance networks ultimately culminates in cardiac-specific autoimmune pathology (Fig. 3B).

Cross-reactivity of cardiac antigens. The pathogenesis of ICI-related myocarditis may predominantly involve tumor-cardiac cross-antigen reactivity. Substantial evidence has indicated that tumor antigens (such as MYH6) share homologous epitopes with myocardial structural proteins (including troponin), which can activate cross-reactive T cells capable of simultaneously targeting both neoplastic and cardiac tissues (79). Experimental validation using immunocompetent A/J murine models has demonstrated that repeated administration of anti-PD-1 antibodies induces myocarditis, with myocardial infiltration of α -myosin-specific CD4⁺ and CD8⁺ T cells identified through TCR analysis. Notably, these cross-reactive T-cell populations have also been detected in the peripheral blood, spleen, mediastinal lymph nodes and cardiac tissues of healthy control mice, suggesting pre-existing autoreactive lymphocytes that become pathogenic upon immune checkpoint inhibition (80). Johnson *et al* (81) revealed that TCR- β clonotypic sequences were highly overlapped between the myocardium, tumors and skeletal muscles of patients with myocarditis, indicating that cross-reactive T cells can mediate multi-organ

damage. Clinically, the frequent co-occurrence of myositis or myasthenia gravis in patients with ICI-related myocarditis aligns with the shared antigenic epitope theory. Particularly, MYHC antigens expressed in both cardiac and skeletal muscles may serve as critical targets for these cross-reactive immune responses (82). Collectively, these findings establish that cross-antigen-driven immune mechanisms underlie the simultaneous multi-organ damage observed in this clinical syndrome (Fig. 4A).

Cellular and molecular mechanisms

T cell-mediated direct injury. T cells serve as central effector cells in ICI-related myocarditis. These cells recognize cardiac antigens and directly attack cardiomyocytes, resulting in tissue damage (83,84). This process is initiated by the binding of TCRs to MHC-antigen complexes presented on the surface of cardiomyocytes, thereby triggering cytotoxic responses.

Role of CD8⁺ T cells. CD8⁺ cytotoxic T lymphocytes serve a pivotal role in immune-mediated myocarditis. They induce cardiomyocyte death through the release of perforin and granzyme B. Perforin facilitates transmembrane pore formation, enabling granzyme B influx and subsequent activation of the caspase-dependent apoptotic cascade, ultimately leading to cardiomyocyte death (85-87). Pathological analyses of myocardial biopsies have revealed notable CD8⁺ T-cell infiltration in focal necrotic regions, with infiltration

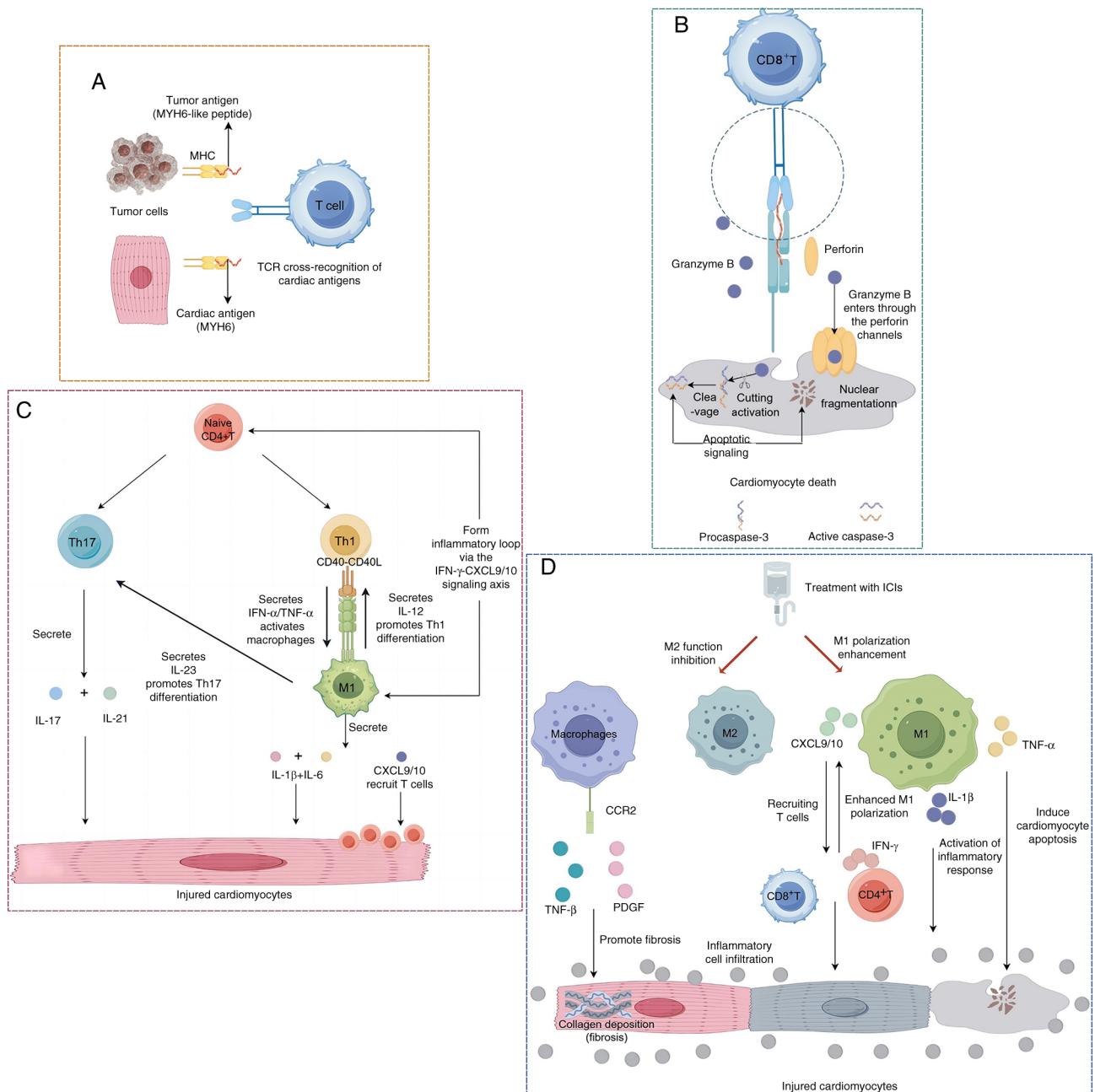


Figure 4. (A) Cross-reactivity of cardiac antigens. (B) Role of CD8⁺ T cells. (C) Role of CD4⁺ T cells. (D) Mechanism of action of macrophages: Infiltration and pro-inflammatory mediator release, antigen presentation, T-cell interactions and macrophage phenotypic plasticity are shown. APC, antigen-presenting cell; ICI, immune checkpoint inhibitor; MYH, myosin heavy chain; TCR, T-cell receptor; Th, T helper; CXCL9, C-X-C motif chemokine ligand 9; MHC, major histocompatibility complex.

levels positively associated with the degree of T-cell clonal expansion (79). Mechanistically, CD8⁺ T cells interact with cardiomyocytes via MHC class I molecules and release cytotoxic mediators; especially when receiving anti-CTLA-4 combined with anti-PD-1 treatment, T-cell activation and proliferation are enhanced, further aggravating myocardial damage (88). Animal models have demonstrated that CD8⁺ T-cell depletion markedly attenuates myocardial inflammation and fibrosis (77). In addition, peripheral blood analyses of patients with ICI-related myocarditis have detected clonally expanded activated cytotoxic CD8⁺ T-cell subsets. A prominent increase is observed in terminally differentiated effector memory CD8⁺ T cells, whereas naïve T cells, central memory T

cells and other effector memory subsets remain unaltered (89). This distinct immunophenotypic shift underscores the critical involvement of CD8⁺ T cells in the pathogenesis of ICI-related myocarditis, with their activation status closely associated with disease severity (Fig. 4B).

Role of CD4⁺ T cells. CD4⁺ T cells drive the inflammatory cascade through cytokine networks in ICI-related myocarditis. Although the number of infiltrating myocardial CD4⁺ T cells is limited, the pro-inflammatory cytokines they secrete, such as IFN- γ and TNF- α , synergistically promote the recruitment and activation of monocytes/macrophages while directly activating apoptotic pathways in myocardial cells, thereby exacerbating tissue damage (90). Clinical

evidence has demonstrated a significant positive association between the density of myocardial CD4⁺ T-cell infiltration and the severity of the inflammatory response, indicating that their regulatory activity is closely associated with the degree of disease progression. In a Ctla-4^{+/-}Pdcd1^{-/-} mouse model, CD4⁺ T cells and macrophages form a self-amplifying inflammatory loop via the IFN- γ -CXCL9/10 signaling axis, leading to persistent deterioration of the inflammatory microenvironment (91). Furthermore, within the inflammatory milieu, cytokines drive the differentiation of CD4⁺ T cells into Th1/Th17 subsets. These subsets recruit neutrophils and activate cytotoxic T cells, thereby contributing to multidimensional mechanisms of myocardial injury (65) (Fig. 4C).

T-cell hyperactivation and autoimmune reactivity. The CTLA-4 protein exhibits superior binding affinity for B7 molecules (CD80/CD86) on APCs compared with CD28. This competitive interaction suppresses CD28-mediated T-cell activation signals, serving as a critical negative regulatory mechanism (52). Anti-CTLA-4 antibodies (such as ipilimumab) disrupt CTLA-4/B7 binding, thereby restoring CD28 co-stimulatory signals and promoting T-cell proliferation, differentiation and cytokine production. The PD-1/PD-L1 pathway, on the other hand, inhibits T-cell function through phosphorylation of the intracellular immune receptor tyrosine-based inhibitory motif, which recruits phosphatases such as SHP-2 to antagonize TCR and CD28 downstream signaling (40). PD-1 inhibitors (such as nivolumab and pembrolizumab) block ligand binding, consequently alleviating T-cell activity suppression and inducing aberrant activation of effector T cells (42,43). Histopathological studies have demonstrated T-cell infiltration within the myocardial tissues of patients of ICI-related myocarditis, with clonal T cell populations showing homology to tumor-infiltrating effector T cells (35,92). Clinical evidence indicates that ICI-induced T-cell clonal expansion drives myocardial tissue targeting, ultimately precipitating myocarditis (81). Mechanistically, ICIs interfere with signaling pathways such as CTLA-4 and PD-1/PD-L1, lowering the T-cell activation threshold and disrupting peripheral immune tolerance, which leads to abnormal T-cell activation and inappropriate immune attacks on myocardial tissue.

Macrophages and inflammatory amplification

Infiltration and pro-inflammatory mediator release. The pathogenesis of ICI-related myocarditis is characterized by prominent macrophage infiltration, as evidenced by histopathological analyses (93). Clinical investigations have demonstrated substantial co-infiltration of CD68⁺ macrophages with CD4⁺/CD8⁺ T lymphocytes within myocardial tissues, suggesting their synergistic role in propagating inflammatory responses (94). Activated M1-type macrophages further amplify the inflammatory response by secreting pro-inflammatory cytokines (TNF- α , IL-1 β and IL-6) and chemokines (CXCL9 and CXCL10), which recruit T cells and other immune cells to infiltrate the myocardial tissue, thereby forming an inflammatory cascade amplification (77). Experimental models utilizing CTLA-4/PD-1-deficient mice recapitulate these findings, showing concurrent infiltration of CD68⁺ macrophages and T cells in myocardial lesions. These

macrophages exacerbate tissue damage through sustained release of IL-1 β and other inflammatory mediators (95). Compared with other types of myocarditis (such as dilated or lymphocytic myocarditis), the myocardial tissue of patients with ICI-related myocarditis shows a notable enrichment of CXCL9⁺/CXCL10⁺ macrophage populations (92).

Antigen presentation and T-cell interactions. Macrophages simultaneously have antigen-presenting function in ICI-related myocarditis. They potentially present cardiac autoantigens (such as α -myosin) via MHC molecules, consequently activating autoreactive T cells and establishing a pathogenic positive feedback loop (96). Experimental models have demonstrated that monocyte-derived CCR2⁺ macrophages within cardiac tissue secrete CXCL9/CXCL10. These chemokines engage CXCR3 receptors on T cells, driving T-cell activation and sustaining a pro-inflammatory microenvironment (91). Furthermore, IFN- γ secreted by activated T cells induces macrophage activation of the JAK/STAT signaling pathway. This reciprocal interaction forms a bidirectional regulatory network that amplifies inflammatory responses (97). These experiments collectively suggest that macrophages and T cells interact and jointly promote the inflammatory response.

Macrophage phenotypic plasticity. Under physiological conditions, M2 macrophages facilitate tissue repair through apoptotic cell clearance and secretion of anti-inflammatory cytokines (such as IL-10) (98). In ICI-related myocarditis, however, this homeostatic balance shifts toward dominance of the pro-inflammatory M1 phenotype (99). Animal model studies have shown that inhibiting macrophage chemotaxis (such as by blocking MCP-1 signaling) or targeting the IFN- γ pathway can reduce the infiltration of pro-inflammatory macrophages and improve myocardial damage (90,100). Single-cell sequencing has further revealed the heterogeneity of macrophage subsets and reported that the CCR2⁺ subset exacerbates pathological myocardial remodeling by activating pro-fibrotic pathways (such as TGF- β signaling), providing a theoretical basis for its potential as a therapeutic target (101). In summary, macrophages in ICI-related myocarditis act both as inflammatory effector cells and as key nodes in immune regulation. Future research should further explore the specific functions of these subsets and the mechanisms of phenotypic conversion to provide a theoretical basis for immune interventions (Fig. 4D).

Other immune cells

Role of B cells. Current evidence does not establish a direct pathogenic role of B cells in ICI-related myocarditis. While murine models demonstrate detectable IgG-secreting plasma cells and anti-myosin autoantibodies, these antibodies likely represent secondary responses to antigen exposure following cardiomyocyte necrosis, given the rare observation of B-cell infiltration in clinical myocardial biopsies (102). Nevertheless, extrapolating from B-cell functions in other subtypes of myocarditis and immune checkpoint regulation of B-cell activity, it may be proposed that B cells could contribute indirectly through antibody-mediated immunity or T-cell collaboration. Studies have shown that in PD-1-deficient mice, there is overactivation of B cells and hypergammaglobulinemia, while PD-1 helps maintain immune homeostasis by inhibiting B-cell proliferation and the secretion of pro-inflammatory

factors such as IL-6 (103,104). These findings suggest that this indirect regulatory mechanism may explain some of the clinical manifestations of humoral immune abnormalities in patients with ICI-related myocarditis.

Role of NK cells. The pathological role of NK cells in ICI-related myocarditis remains incompletely defined. Studies have indicated that NK cells express immune checkpoint molecules, including PD-1 and CTLA-4, and their cytotoxic activity may be modulated by checkpoint signaling pathways. Prolonged antigenic stimulation can upregulate PD-1 expression on NK cells, leading to impaired cytotoxic function (105,106). Consequently, ICI therapy may reverse this inhibitory state, potentiating NK cell cytotoxicity and the release of pro-inflammatory mediators such as IFN- γ , thereby contributing to the pathogenesis of myocarditis (107). Nevertheless, existing evidence has not confirmed direct infiltration of NK cells into myocardial tissue or their role in mediating cardiomyocyte injury.

Genetic and microenvironmental determinants

Genetic predisposition. The HLA-DQ8 allele, encoding a specific MHC class II heterodimer, exhibits strong associations with autoimmune pathologies including type 1 diabetes mellitus and celiac disease. Its molecular structure markedly increases the risk of ICI-related myocarditis in carriers by enhancing autoantigen presentation and T-cell activation (108). This histocompatibility complex potentiates autoimmune responses through structural facilitation of self-antigen presentation and subsequent CD4⁺ T-cell activation, conferring elevated risk for ICI-related myocarditis in genetically predisposed individuals (109). Mechanistic validation comes from murine models engineered with humanized HLA-DQ8 expression on an MHC class II-deficient background. These preclinical models develop severe concurrent myocarditis and myositis following anti-PD-1 therapy, recapitulating the human disease phenotype (110). This evidence suggests that specific genetic backgrounds, such as HLA-DQ8, may significantly increase the susceptibility to ICI-related myocarditis (111).

Thymic dysfunction. Patients with TETs exhibit a markedly elevated incidence of myocarditis following ICI therapy. These individuals frequently develop severe arrhythmia and myositis, complications that may progress to respiratory failure or fatal outcomes (111-113). The elevated levels of serum anti-acetylcholine receptor antibodies in this population suggest that thymus-related autoimmune disorders are one of the causes of the disease (114).

Mechanistically, the thymus regulates T-cell development and central tolerance via CTLA-4 and PD-1 signaling. In patients with TETs, thymic structural disruption creates dual pathological consequences: i) Absence of cardiac-specific antigens (such as MYHCA) in the thymic medulla permits autoreactive T cells to evade negative selection (16); ii) the tumor microenvironment may induce the formation of T-cell subsets with potential self-targeting tendencies. After ICI treatment relieves immune inhibitory signals, these T-cell subsets become activated and breach immune tolerance, leading to an attack on cardiac tissue (115). This thymic dysfunction, in combination with the effects of ICI therapy, ultimately results in a markedly increased risk of developing myocarditis.

Gut microbiome. The molecular mimicry between peptides derived from gut commensal microbiota and cardiac myosin (such as MYH6) may activate cardiac-specific Th17 cells, thereby triggering autoimmune responses against myocardial tissue. Studies have suggested that such cross-reactive peptides drive ICI-related myocarditis by promoting Th17 cell activation and cross-recognition of tumor/microbial antigens. Furthermore, variations in gut microbiota composition among patients may modulate these immune responses, thereby influencing disease susceptibility (89,116).

Other aspects

Myocardial electrophysiology and conduction system injury. ICI-related cardiac electrophysiological abnormalities can induce arrhythmias through multiple pathways. Primarily mediated by inflammatory responses (myocarditis), these abnormalities trigger myocardial fibrosis and localized scar formation. Such anatomical remodeling establishes a pathological substrate for reentrant arrhythmias (such as atrial fibrillation and ventricular fibrillation) through the creation of ectopic electrical foci or conduction block zones (117,118). The electrophysiological regulation of the cardiac conduction system is intrinsically linked to macrophage functionality through electrical coupling mediated by connexin 43 (Cx43) with cardiomyocytes (119). Hulsmans *et al* (119) demonstrated that macrophage-specific Cx43 ablation causes atrioventricular conduction delays, whereas CD11b deficiency induces progressive atrioventricular blocks, implicating macrophage-dependent Cx43 signaling in both physiological conduction and arrhythmogenesis. These synergistic mechanisms collectively amplify arrhythmia susceptibility during ICI therapy.

Protective effects of estrogen. The sex disparity (the incidence or severity is higher in women) observed in ICI-related myocarditis incidence may be attributed to the estrogen-estrogen receptor (ER) β -mesencephalic astrocyte-derived neurotrophic factor (MANF) signaling axis. Mechanistic studies have demonstrated that estrogen activates ER β signaling, upregulating MANF. This neurotrophic factor confers cardioprotection through two key mechanisms: Suppressing myocardial T-cell infiltration and inhibiting pro-inflammatory cytokine release (including IFN- γ and TNF- α) (120,121).

Experimental data from female murine models have revealed that ICI treatment induces 17- β -estradiol depletion, concomitant with reduced cardiac MANF expression, which exacerbates myocarditis progression. Pharmacological intervention with exogenous estrogen or ER β -selective agonists restores MANF levels, markedly attenuating myocardial inflammation and fibrotic remodeling (122). These findings elucidate the molecular basis of estrogen-mediated protection and provide a rationale for sex-specific therapeutic strategies in ICI-associated cardiac toxicity.

5. Future directions

Biomarker development and diagnostic technologies. Advancements have notably improved early diagnostic strategies for ICI-related myocarditis. Myocardium-specific

Table I. Graded treatment strategies for ICI-related myocarditis.

Severity	First-line treatment	Second-line treatment	Monitoring and key actions
Mild (Grade 1): Suspected or confirmed, asymptomatic with cardiac biomarker elevation or ECG changes	1) Hospitalization for monitoring. 2) Initiation of corticosteroids: Prednisone 1-2 mg/kg/day orally (or methylprednisolone IV equivalent). 3) Discontinuation of ICI therapy.	If symptoms develop or biomarkers worsen, escalation to moderate/severe treatment protocol.	Serial monitoring: -Troponin -ECG -Echocardiography (LVEF) Decision to resume ICI is highly individualized if symptoms resolve and biomarkers normalize.
Moderate to severe (Grade 2-3): Significant symptoms (e.g., chest pain, dyspnea), biomarker elevation, LVEF dysfunction or arrhythmias	1) Immediate hospitalization (often to cardiac unit). 2) High-dose corticosteroids: Methylprednisolone 1-2 mg/kg/day IV. 3) Permanent discontinuation of ICI.	If no clinical improvement within 24-72 h of high-dose steroids: -Add second-line immunosuppressants: • Mycophenolate mofetil • Antithymocyte globulin (ATG) • Abatacept (especially for fulminant cases) -CONTRAINDICATED: Infliximab (associated with increased cardiovascular mortality and heart failure).	Intensive monitoring: -Frequent troponin, ECG -Echocardiography -Assess for multi-organ ir AEs (e.g., myositis, MG). -Manage heart failure/arrhythmias as per standard guidelines.
Life-threatening (Grade 4): Hemodynamic instability, cardiogenic shock, lethal arrhythmias	1) Admit to ICU. 2) Pulse-dose corticosteroids: Methylprednisolone 1,000 mg/day IV. 3) Add IV Immuno-globulin (IVIG). 4) Permanently discontinue ICI.	Initiate urgently (concurrently or if no immediate response): -Second-line agents: ATG or Abatacept. -Mechanical circulatory support: Consider VA-ECMO for cardiogenic shock. -Temporary pacing for high-grade heart block. -Heart failure/arrhythmia support.	ICU-level monitoring: -Hemodynamics (e.g., Swan-Ganz catheter) -Continuous telemetry -Multi-organ function support.

Based on Management of immune-related adverse events in patients treated with immune checkpoint inhibitor therapy: ASCO guideline update (130). 2022 ESC Guidelines on cardio-oncology developed in collaboration with the European Hematology Association, the European Society for Therapeutic Radiology and Oncology and the International Cardio-Oncology Society (131). ATG, antithymocyte globulin; ECG, electrocardiogram; ECMO, extracorporeal membrane oxygenation; ICI, immune checkpoint inhibitor; IV, intravenous; LVEF, left ventricular ejection fraction.

autoantibodies (such as anti-myosin antibodies) and TCR repertoire analysis have the potential for early diagnosis of ICI-related myocarditis. Notably, T-cell infiltration and upregulation of PD-L1 expression has been detected in the myocardial tissue of patients with ICI-related myocarditis, suggesting a T cell-mediated immune injury mechanism (95). Additionally, the combined detection of myocardial-specific microRNAs (miRs), such as miR-208a, and cTnI enhances diagnostic sensitivity, as miR-208a is released earlier than cTnI alone (123). Furthermore, optimization of imaging diagnostics and artificial intelligence-assisted systems may address current

diagnostic limitations (124). Future efforts should integrate multi-omics biomarker datasets with dynamic monitoring platforms to establish non-invasive, high-sensitivity diagnostic frameworks.

Innovative targeted therapeutic strategies. The standard first-line treatment for ICI-related myocarditis involves high-dose corticosteroids (such as methylprednisolone, 1,000 mg/day for 3 days), followed by a tapering regimen over several weeks. For steroid-refractory cases, immunosuppressants such as abatacept, mycophenolate mofetil or infliximab

may be employed. Early treatment within 72 h is critical for improved outcomes (125).

Advances in targeted therapeutic strategies for ICI-related myocarditis have emerged as a critical research focus. Current efforts have concentrated on three principal domains: i) Immune checkpoint regulation: By antagonizing CTLA-4 or modulating downstream PD-1 molecules (such as PI3K/AKT/mTOR), these strategies aim to balance immune activation and myocardial protection (126). ii) Cytokine-targeted intervention: Implementation of IL-6 receptor antagonists (tocilizumab) and TNF- α inhibitors may alleviate the onset of inflammation. Clinical evidence has indicated that survival outcomes are enhanced in critically ill patients when combined with extracorporeal membrane oxygenation support (127). iii) Development of specific monoclonal antibodies: Antibody drugs targeting myocardial-specific antigens, such as cTns, may reduce nonspecific attacks, although this technology is still in the preclinical research phase (89). Additionally, innovative combination therapies have garnered attention. For example, low-dose corticosteroids combined with JAK/STAT pathway inhibitors (such as baricitinib) can synergistically suppress hyperactivated immune responses while reducing the toxicity associated with conventional treatments (128). Nevertheless, current evidence remains predominantly derived from limited clinical cohorts, underscoring the imperative for large-scale randomized controlled trials to validate safety and efficacy profiles. Future investigations should prioritize multi-omics-driven target discovery and advanced drug delivery platforms to optimize myocardial-specific immunomodulatory precision.

Multidisciplinary collaboration. Rechallenge with ICIs after immune-related myocarditis remains a controversial clinical issue. Rechallenge may be considered under stringent conditions: Complete resolution of myocarditis symptoms, normalization of troponin and NT-proBNP, resolution of imaging findings and consensus by an MDT. Emerging guidelines suggest cautious use and rechallenge is typically avoided unless no alternatives exist (125).

The management of ICI-related myocarditis necessitates MDT collaboration involving cardiology, oncology and immunology to optimize early detection and personalized interventions. Real-time coordination between oncologists and cardiologists enables balanced therapeutic decision-making, including ICI dose adjustments or co-administration of immunomodulatory agents to mitigate cardiotoxicity without compromising antitumor efficacy (Table I) (129-131). In the future, standardized collaborative processes need to be established to improve prognosis.

6. Conclusion

ICI-related myocarditis represents a critical challenge in cancer immunotherapy. While ICIs have revolutionized survival outcomes in malignancies, their cardiovascular toxicity substantially limits their clinical utility. Current diagnostic strategies integrating biomarker profiling, cardiac imaging and histopathological confirmation remain constrained by delayed detection and suboptimal specificity.

Current research is focused on three core issues: i) The pathophysiological mechanisms, particularly the role of T-cell clonal expansion and the cross-reactivity between myocardial antigen epitopes, which remain to be fully elucidated; ii) the absence of validated predictive biomarkers and risk stratification tools; iii) existing stratified treatment strategies lack evidence-based support. Future research is needed in the following areas: Firstly, systematic biology methods should be employed to analyze dynamic changes in the immune microenvironment, and single-cell sequencing techniques should be used to reveal the clonotype characteristics of myocardium-specific TCRs. Secondly, multi-omics predictive models integrating genomics, proteomics and metabolomics should be developed. Additionally, interdisciplinary research platforms spanning oncology, cardiology and immunology should be established. Particular emphasis should be placed on cohort studies and biobank development, as these will provide crucial support for optimizing diagnostic and therapeutic pathways. With the ongoing expansion of indications for immunotherapy, further elucidating the molecular mechanisms of ICI-related myocarditis and establishing prevention and treatment strategies will become key directions for future research. Current research must prioritize elucidating risk stratification mechanisms to achieve a precise equilibrium between therapeutic efficacy and cardiotoxicity.

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Competing interests

The authors declare that they have no competing interests.

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