HUMAN VACCINES & IMMUNOTHERAPEUTICS
2025, VOL. 21, NO. 1, 2512645
https://doi.org/10.1080/21645515.2025.2512645

Taylor & Francis
Taylor & Francis Group

REVIEW



# A review of immune checkpoint inhibitor-associated myocarditis: Epidemiology, pathogenesis, and biomarkers

Le Xu[a*], Yukai Chen[a*], Lin Xiong[b], Yang Shen[a], Zhuolin Zhou[a], Siyu Wang[a], and Ximing Xu[a]

[a]Cancer Center, Renmin Hospital of Wuhan University, Wuhan, China; [b]Pathology Department, Renmin Hospital of Wuhan University, Wuhan, China

**ABSTRACT**

Immune checkpoint inhibitor (ICI) have demonstrated efficacy in treating various cancers by modulating the immune system, but this can lead to immune-related adverse events (irAEs), including myocarditis. ICI-associated myocarditis is a rare but highly lethal irAE with a short mean time to onset, and difficult to diagnose early due to nonspecific symptoms and lack of biomarkers. This review highlights the need for improved recognition and management of ICI-associated myocarditis, summarizing recent advances in immunology, pathology, and biomarker research. We discuss the epidemiology, clinical features, immunological mechanisms, and roles of biomarkers in diagnosis and risk stratification. Traditional biomarkers like cTnI and hs-cTnT are sensitive but lack specificity, while emerging biomarkers like miR-155 show tissue specificity. Inflammatory markers such as NLR and CRP aid prognosis but have limited diagnostic value.

**ARTICLE HISTORY**
Received 5 December 2024
Revised 15 May 2025
Accepted 25 May 2025

**KEYWORDS**
Biomarkers; irAEs; immune checkpoint inhibitors; myocarditis; pharmacology

## Introduction

Immune checkpoint inhibitors (ICI) are a class of therapeutic agents that enhance anti-tumor immunity by stimulating T-lymphocyte activation and improving tumor recognition. The most commonly used ICI target programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte – associated antigen 4 (CTLA-4) pathways. These immune checkpoints play essential roles in maintaining self-tolerance and preventing autoimmunity. However, malignant tumors can hijack these inhibitory pathways to evade immune surveillance, thereby facilitating tumor progression and metastasis. By blocking these co-inhibitory signals, ICI restore T cell cytotoxic function and reinvigorate anti-tumor responses.

The widespread use of ICI has significantly improved survival outcomes in patients with advanced and refractory cancers. Despite the remarkable benefits, ICI are associated with various immune-related adverse events (irAEs), such as colitis, pneumonia, hepatitis, thyroid dysfunction, and myocarditis (Figure 1b).[1–4] While colitis is the most common but least lethal irAE, myocarditis is a rare but highly lethal complication, drawing increasing attention.[5] Myocarditis caused by ICI often results from immune activation leading to autoimmune attacks on cardiac tissue, causing severe cardiac dysfunction.

ICI-associated myocarditis (ICI myocarditis) is estimated to occur in approximately 1.14% of patients, but it carries a strikingly high mortality rate of up to 50%.[6] Due to its unpredictable onset and fulminant course, early diagnosis and prompt intervention are crucial for improving patient outcomes. However, effective early detection methods are currently lacking, and many cases are identified only at advanced stages, resulting in poor prognosis. As the clinical use of ICI continues to expand, understanding the pathophysiological mechanisms behind ICI-associated myocarditis has become increasingly urgent.

This review aims to explore the potential mechanisms underlying ICI-associated myocarditis, focusing on how ICI trigger immune responses that lead to cardiac autoimmunity. In addition, we highlight the importance of identifying reliable biomarkers – such as conventional cardiac markers, inflammatory and immune-related indicators, and emerging molecular signatures – for early diagnosis and risk prediction. Finally, current therapeutic approaches and future perspectives in the management of ICI-associated myocarditis will also be discussed.

## Immune checkpoint inhibitors: mechanisms of action and role in immune tolerance

ICI are monoclonal antibodies that enhance antitumor immunity by blocking inhibitory receptors on T cells such as CTLA4 and PD1 or their ligands (PDL1). In 2018, James Allison and Tasuku Honjo received the Nobel Prize in Physiology or Medicine for elucidating the critical roles of CTLA4 and PD1 in immune checkpoints, laying the foundation for subsequent development of ICI. Since the mid2000s, monoclonal antibodies targeting CTLA4 and PD1/PDL1 have been developed and approved (Table 1).

The human body has a strong adaptive immune system capable of eliminating most pathogens, such as bacteria and viruses, through cellular and humoral immunity. Therefore, the immune system must maintain self-tolerance to prevent the immune system from damaging the body's normal tissues. This task is accomplished through central tolerance, where

**CONTACT** Ximing Xu doctorxu120@aliyun.com Cancer Center, Renmin Hospital of Wuhan University, Wuhan 430060, China.
*These authors contributed equally to this work.

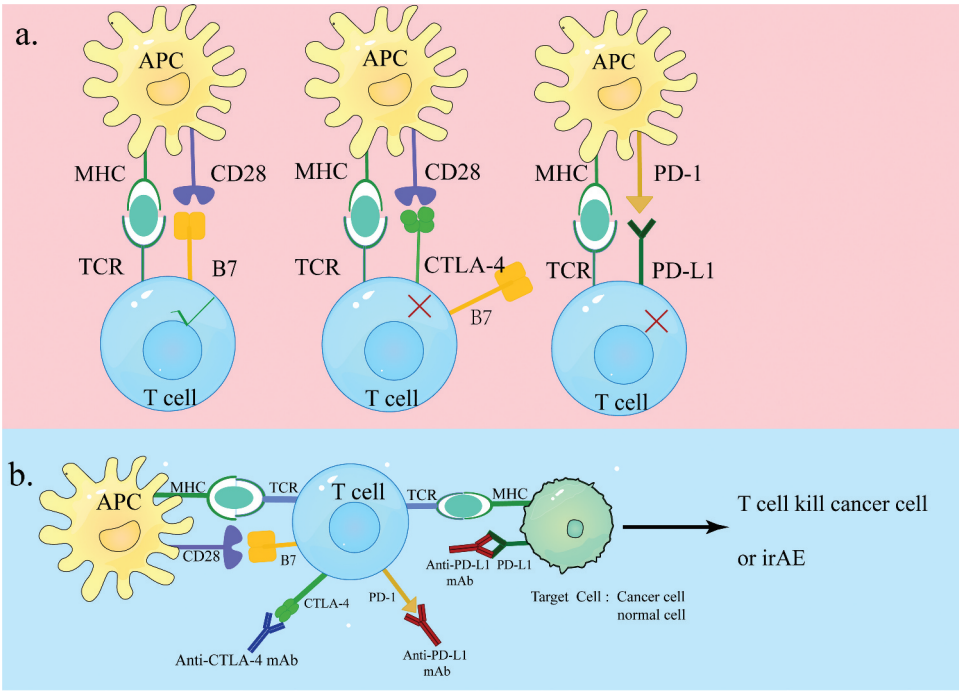


**Figure 1.** (a) T cells are not activated when co-stimulation is blocked. (b) ICI can reinvigorate T cells to kill tumor cells and can also lead to irAes.

**Table 1.** ICI approved by the FDA.

| Major ICI | Target | FDA Approval Date | Mechanism |
|---|---|---|---|
| Ipilimumab (Yervoy) | CTLA4 | March 25, 2011 | Blocks CTLA4 to enhance CD28–B7 costimulation |
| Pembrolizumab (Keytruda) | PD1 | September 4, 2014 | Inhibits PD1 binding to PDL1/PDL2 |
| Nivolumab (Opdivo) | PD1 | 2014(first approval) | Inhibits PD1 binding to PDL1/PDL2 |
| Atezolizumab (Tecentriq) | PDL1 | 2016 | Blocks PDL1 interaction with PD1 |

lymphoid organs are selected to eliminate T cells that can capture their own antigens via negative selection, and peripheral tolerance, where peripheral lymphocytes acquire tolerance to their own or exogenous antigens via a variety of mechanisms.[7,8] PD-1 and CTLA-4 are critical immune checkpoint proteins in the maintenance of peripheral immune tolerance. Activation of resting T cells requires three distinct signals: first, engagement of the T cell receptor (TCR) with peptide – MHC complexes on the antigen-presenting cell (APC); second, costimulation via CD28 on the T cell binding to B71 (CD80)/B72 (CD86) on the APC; and third, cytokine signaling – such as interleukin 12 (IL12) – which drives full proliferation, differentiation and acquisition of effector function. Notably, blockade of the CD28–B7 interaction alone is sufficient to prevent T cell activation (Figure 1a).

CTLA-4 is a transmembrane receptor on T cells, which is homologous to CD28 but can capture B7 molecules with stronger competitiveness and then transmit inhibitory signals to T cells.[9,10] The extracellular role of CTLA-4 is equally significant, as CTLA-4 can capture its ligands from opposing cells via trans-endocytosis and degrade them intracellularly, resulting in impaired co-stimulation through CD28. This mode of action was previously only thought to exist in FOXP3+ Treg cells, but it was later found that the same mode of action also exists on effector T cells, suggesting a broad immunosuppressive effect of CTLA-4.[11,12] CTLA-4-deficient mice develop lymphoproliferative diseases with lymphocytic infiltration and tissue destruction in multiple organs, particularly myocarditis, indicating the critical role of CTLA-4 in maintaining immune tolerance.[13] PD-1 is also expressed during T cell activation and interacts with PD-L1 or PD-L2 to counter T cell co-stimulatory signals.[14] PD-L1 is abundantly expressed in the heart, skeletal muscle, placenta, and lung, but in the thymus, spleen, kidney, and liver, the expression is weak, and it is not expressed in the brain, colon, and small intestine.[15] By contrast, the expression of PD-L2 is more restricted, being expressed primarily in the pancreas, spleen, liver, and B cell populations.[16,17] The deletion of PD-1/PD-L1 leads to an increase in pro-inflammatory factors, especially IFN-γ (Interferon gamma). However, IFN-γ in turn elevates the expression of PD-1 ligands as a way to reduce the damage caused by the inflammatory response to the body, which is an important negative feedback regulation[18,19] (Figure 2). Cancer cells can express both PD-L1 and PD-L2, which helps them avoid immune cell attacks. Seventy-one percent of 62 lung cancer specimens from Egypt's Mansoura Medical College Hospital tested positive for PDL-1, with approximately 59.1% being highly expressed.[20] Immunohistochemical staining of PD-L1 and PD-L2 expression in the cytoplasm of paraffin-embedded tumor samples revealed that 96 (64.0%) of 150 ESCC patients included in Taipei Veterans General Hospital had PD-L1 overexpression and 63 (42.0%) had PD-L2 overexpression. There was a correlation between PD-L1 and PD-L2 expression, and

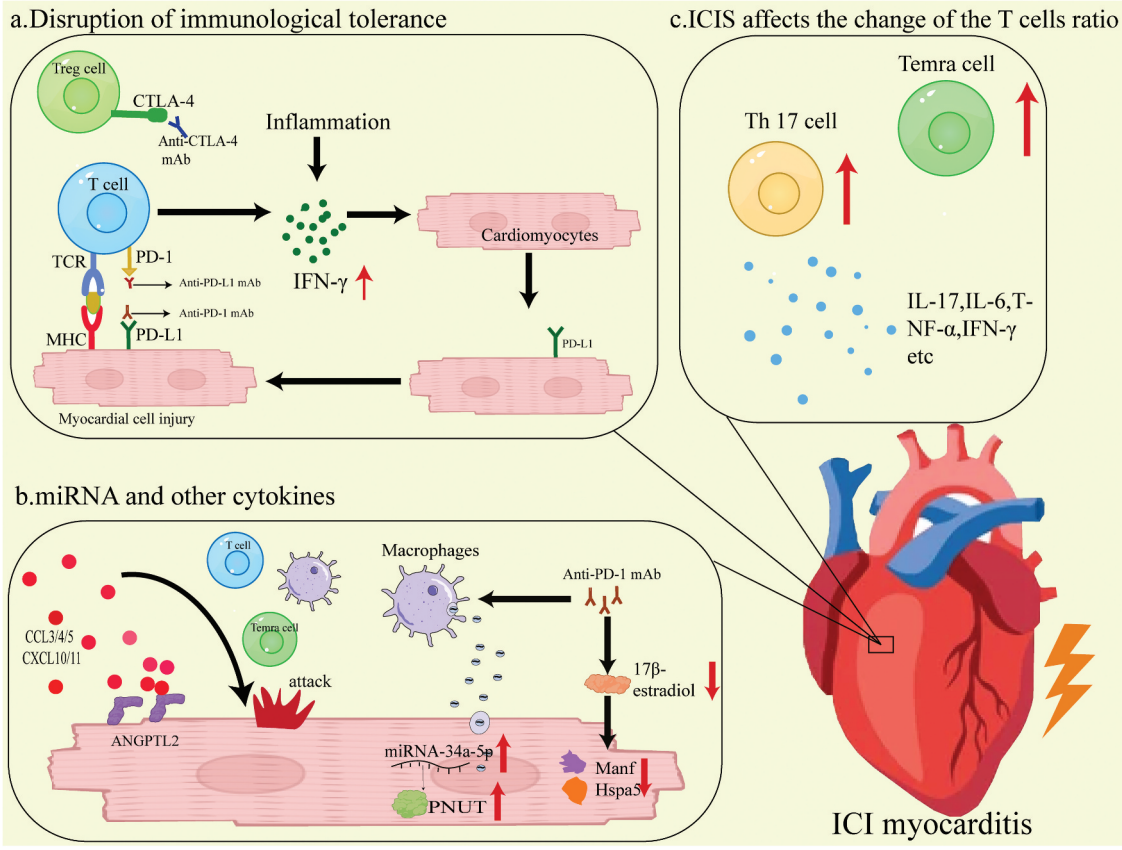


**Figure 2.** Mechanism of ICI myocarditis. (a) IFN-γ is a pro-inflammatory factor, however, exhibits anti-inflammatory effects in the myocardium. Elevated levels of IFN-γ induce PD-L1 overexpression in cardiomyocytes when PD-L1 is deficient or when there is inflammation. This unique regulatory effect protects cardiomyocytes from T cell attack. PD-L1 inhibitors block this negative feedback regulation, leading to ICI myocarditis. Treg cells are suppressed by ICI and therefore lose their ability to suppress effector T cells. (b) Exosomes from ICI-treated macrophages can induce senescence-associated injury in cardiomyocytes by modulating the miR-34a-5p/PNUTS signaling pathway. ICI reduces serum 17β-estradiol concentrations, leading to downregulation of MANF/HSPA5 in cardiomyocytes. MANF and HSPA5 are essential for attenuating myocardial inflammation in ICI myocarditis. ANGPTL2 promotes the expression of chemokines such as CCL3/4/5 and CXCL10/11 leading to T cell recruitment. (c) The expansion of Temra cells, Th17 cells, etc. Was found on patients with ICI myocarditis, and they cause damage to cardiomyocytes through multiple pathways.

these co-inhibitory signals counterbalance each other with co-stimulatory signals from CD28 to maintain immune homeostasis.[21] By blocking the PD-1 or CTLA-4 pathways, T cells that were previously suppressed are reactivated to target and eliminate cancer cells. ICI have demonstrated notable efficacy in the treatment of certain types of cancer, however, this effect disrupts immune tolerance and causes a variety of irAEs, including colitis, pneumonia, and skin inflammation, the most concerning of which is ICI-associated myocarditis. Although there is evidence that patients who develop irAE may have better treatment outcomes, ICI-associated myocarditis must be an exception due to its high mortality rate.[22]

## ICI-associated myocarditis: epidemiology and clinical features

### *Epidemiology and pathology*

Pathology underlying immune checkpoint inhibitor ICI myocarditis is typified by the inflammation of lymphoid tissue cells, an augmented CD68/CD3 ratio, an elevated number of PD-L1+ macrophages and myocytes, patchy T cell-dominated lymphocyte infiltration in the myocardium, and associated myocardial damage. The severity of ICI myocarditis is stratified into high-grade and low-grade subtypes, based on the density of the inflammatory infiltrate, with the former presenting with a more aggressive clinical course and the latter displaying a more chronic clinical course.[6,23,24] The vast majority of ICI myocarditis occurs within a few months after the first or second use of ICI, and the incidence and mortality rate of ICI myocarditis compared with other immune adverse events are unclear, although some studies have reported results.[25] The more widely used data are from an eight-center retrospective study reporting a 1.14% incidence of ICI myocarditis with a median time to onset of 34 days, and data from a large network of healthcare organizations showed that the incidence of myocarditis in 5518 cancer patients treated with at least one cycle of ICI was 2.1%.[6,26,27] The incidence rates obtained from various studies vary greatly, which is likely influenced by the type of cancer, treatment regimen, and ethnicity. However, it is almost certain that the incidence and mortality rate of combination ICI therapy are much higher than those of monotherapy. With the widespread use of ICI, the trend has become evident that the cases and incidence of ICI-cardiomyopathy are increasing. The majority of patients with ICI myocarditis are currently concentrated in melanoma and non-small cell

lung cancer, owing to the fact that ICI therapies are mostly used for these two types of cancer. Patients with other cancer types are occasionally found, and ICI myocarditis is also found in patients with Hepatocellular carcinoma (HCC). Therefore, the development of a cost-effective and rapid biomarker is of great importance for the prevention and treatment of myocarditis.[28–31] Unlike fulminant myocarditis, ICI-myocarditis is more common in women, which is surprising since non-ICI-related myocarditis occurs more prevalent in men. The elderly population is more prone to ICI myocarditis, with a high incidence of ICI myocarditis around age 65.[32–36]

### *Clinical features*

Early manifestations of immune checkpoint inhibitor (ICI)-associated myocarditis typically occur within the first month of therapy and often present with nonspecific symptoms such as fatigue, dyspnea, and chest discomfort. In approximately 30.3% of cases, these are accompanied by neuromuscular complaints, including myalgia or proximal muscle weakness.[37] Cardiac-specific presentations may include chest pain (reported in 13%–28% of cases), palpitations (5%–33%), and electrocardiographic abnormalities – such as ST – T wave changes, conduction delays, or varying degrees of atrioventricular block – which are observed in up to 85.1% of patients. Notably, elevated high-sensitivity troponin levels are almost universally present at the time of diagnosis, even when echocardiography demonstrates preserved left ventricular function (*38*). Furthermore, approximately 10% of cases are incidentally identified during routine biomarker testing or electrocardiographic monitoring in asymptomatic patients.[37]

Although the majority of cases present acutely, delayed-onset myocarditis – emerging several weeks to months following ICI initiation – is increasingly recognized.[38] In advanced stages, clinical deterioration may manifest as overt heart failure with reduced ejection fraction, hypotension, or cardiogenic shock necessitating inotropic support or mechanical circulatory assistance. Life-threatening ventricular arrhythmias, including ventricular tachycardia and ventricular fibrillation, are reported in approximately 13% of cases. High-grade AV block is observed in up to 25% of patients, frequently requiring permanent pacemaker implantation.[37,39,40] Persistent conduction system abnormalities and ongoing myocardial injury may be observed even after initial treatment, indicating the need for long-term monitoring and management.

## Prognosis of ICI-associated myocarditis: risk factors and outcomes

Although immune checkpoint inhibitor (ICI)-associated myocarditis is rare, with an incidence of about 1%, it has a high mortality rate of 40%–50%, as consistently reported in multiple studies. A pharmacovigilance study of 122 patients found a 50% mortality rate, with myocarditis typically occurring around 30 days after starting ICI treatment. A multicenter retrospective study similarly showed that all affected patients experienced grade 5 adverse events, highlighting the severe clinical course of this condition.[41,42] Combination ICI therapy, such as ipilimumab plus nivolumab, carries a higher risk of fatal myocarditis than monotherapy, likely due to overlapping immune-related toxicities like concurrent myositis.[5,43] Although it represents a small portion of immune-related adverse events, ICI-associated myocarditis accounts for nearly one-third of ICI-related deaths.

Emerging evidence suggests that preexisting comorbidities such as diabetes, hypertension, or underlying cardiovascular disease may predispose patients to ICI-related myocarditis, although further large-scale, prospective studies are needed to validate these associations.[44,45] Several prognostic indicators have been proposed for early risk stratification. Elevated high-sensitivity cardiac troponin levels at presentation (median peak troponin T: 0.81 ng/mL) reflect significant myocardial injury and correlate with both acute disease severity and long-term cardiovascular outcomes. Involvement of the cardiac conduction system – most notably, complete heart block (CHB), which occurs in approximately 15% of cases – is associated with a markedly increased risk of death (hazard ratio 7.41).[46,47] Additionally, left ventricular systolic dysfunction serves as an independent adverse prognostic factor, with a mean ejection fraction of 43% at onset.[46]

Despite the prompt initiation of immunosuppressive therapy, including corticosteroids, the in-hospital fatality rate remains high. While some reports suggest that high-dose corticosteroids may improve outcomes in severe presentations, others have demonstrated increased rates of major adverse cardiac events (MACE) among patients receiving low-dose steroid regimens. In a cohort of 35 patients, 89% received corticosteroids, yet low-dose therapy was significantly associated with higher MACE incidence.[6,48] MACE was defined as a composite outcome including cardiovascular death, cardiac arrest, cardiogenic shock, and hemodynamically significant CHB. These findings underscore the urgent need to refine immunosuppressive strategies – considering optimal steroid dosing, duration, adjunctive immunosuppressants, and their potential impact on anticancer efficacy.

Long-term cardiovascular morbidity among survivors is also substantial. In a matched case-control cohort (76 myocarditis patients vs. 200 controls), only 67% of myocarditis survivors were free from new cardiovascular events – defined as atrial fibrillation, stroke, myocardial infarction, or heart failure – at two years, compared to 86.8% in controls ($p < .001$). During follow-up, 12% of survivors developed atrial fibrillation and 26% developed new-onset heart failure. Moreover, two-year cardiovascular survival was significantly lower in the myocarditis group compared to controls (93.4% vs. 99.3%, $p = .003$), highlighting a sustained elevated cardiovascular risk even after apparent clinical recovery.[46]

## Mechanism of ICI-associated myocarditis

### *Loss of immune checkpoint regulation*

The mechanism of ICI-associated myocarditis is not clear, but most believe it is due to the disruption of immune homeostasis by ICI, resulting in T cell-based immune damage. As previously mentioned, PD-1 binding to its ligand can maintain the homeostasis of the immune system

by activating intracellular signaling pathways to inhibit the activation of immune cells, thereby reducing the secretion of antibodies and cytokines by immune cells and even depleting them. The PD-1 pathway is an important barrier that protects cardiomyocytes from T cell destruction, and inhibiting ICI leads to autoimmune disease caused by the T cell attack on cardiomyocytes. Pdcd1$^{-/-}$ mice develop autoimmune dilated cardiomyopathy and produce high titers of autoantibodies against cardiac-specific 30-kDa proteins, and another study showed MRL-Pdcd1$^{-/-}$ mice develop lethal myocarditis and high titers of anti-CM autoantibodies.[49–51] The deletion of both PD-L1 and PD-L2 causes severe inflammatory infiltration of the heart in mice.[52–54] All of these studies point to a protective role of the PD-1 pathway for the heart. Surprisingly, however, mice from different genetic backgrounds seem to respond differently to PD-1 pathway blockade, with BALB/C and MAL mice inducing myocarditis in the presence of PD-1 deletion, yet C57BL/6J and C57BL/10J mice having difficulty inducing myocarditis.[55] A more critical point of the blockade of the PD-1 pathway is the disruption of the FOX3$^{+}$ Treg/PD-L1 axis, a negative feedback regulation of the heart.[18] Using PD-1 inhibitors followed by PD-L1 inhibitors resulted in more severe myocardial damage than using PD-L1 inhibitors followed by PD-L1 inhibitors, implying a protective role for the negative feedback pathway in myocarditis.[56] IFN-γ is elevated during the development of ICI myocarditis, and IFN-γ induces PD-L1 expression in cardiomyocytes reducing myocardial cell injury. High expression of PD-L1 has been observed by staining cardiomyocytes from patients with ICI myocarditis, but ICI blocked the negative feedback pathway[21,57](Figure 2 a). Incidentally, the combination of radiotherapy with anti-PD-1 can increase the incidence of ICI-associated myocarditis.[58] Blockage of the CTLA-4 signaling pathway is also an important factor in the development of ICI myocarditis. Wang et al. reported that CTLA-4$^{-/-}$ mice are more prone to ICI myocarditis compared to PD-1$^{-/-}$ mice, which is consistent with other studies. CTLA-4 deletion activated CD4/CD8+ T cells, causing them to attack cardiomyocytes. CTLA-4 deficiency for the regulation of Treg cells is a non-negligible part of the immune homeostasis of the organism.[50,59,60] CTLA-4 is more frequently expressed on Treg cells in addition to activated T lymphocytes. Treg cells are an immunosuppressive subset of CD4+ T cells, and 80% of Treg cells are thymus-derived, suggesting that Treg cell formation is associated with central tolerance. Treg cell-mediated T cell suppression is a third mechanism for maintaining self-tolerance in addition to negative selection and co-inhibitory pathways.[61,62] As previously discussed, CTLA-4 captures and degrades ligands on target cells through extracellular endocytosis, especially the downregulation of CD80 and CD86 expression on dendritic cells, which is currently thought to be the most prominent mechanism by which Treg cells exert their inhibitory effects[63] (Figure 2a). The cytokinesis of CTLA-4-dependent Treg cells may promote PD-L1-dependent co-inhibition in addition to limiting CD80/CD86 co-stimulation. Murat et al. discovered that soluble and Treg cell-expressed CTLA-4 could enhance PD-L1 co-inhibition by depriving CD80 via CD80 blockade or cytokinesis, respectively, and releasing free PD-L1 from the cis-CD80/PD-L1 heterodimer on DC cells.[64] This suggests that the PD-1 and CTLA-4 pathways reinforce each other and that combining the two drugs may have an enhanced antitumor effect, though such a therapeutic approach increases the risk of irAE.

### Activation of autoreactive T cells

For autoimmune diseases, whether humoral or cellular immunity, the antigen targeted is crucial. But unfortunately, for ICI myocarditis, there is no universally accepted answer. ICI can release T lymphocyte activity to disrupt immune tolerance, but the development of ICI myocarditis is predicated on the ability of T cells to attack cardiomyocytes. Based on this scenario, there are several possibilities[1]: the presence of the same antigen in the tumor as in the myocardium,[2] the presence of T cells targeting the myocardial antigen already in the body[3] the same T cell receptor targeting the tumor antigen and a different but homologous muscle antigen. In a 2017 case report, high levels of muscle-specific antigens (junctional proteins and troponin) were observed in the tumors of two patients with melanoma, and T cell receptor next-generation sequencing revealed these two patients shared high-frequency T cell receptor sequences among cardiac, skeletal muscle, and tumor infiltrates. T cells are activated and cloned in large numbers by tumor-expressed antigens and subsequently attack cardiac myocytes causing myocarditis.[65,66] The absence of α-MyHC in the human thymus has been demonstrated as early as 2010, implying central tolerance to α-myosin may be inherently lacking in humans, which could also explain why the heart is often the primary target once immune homeostasis is disrupted.[67,68] α-MyHC is also the most commonly used drug in the creation of autoimmune myocarditis models. Axelrod et al. first demonstrated the presence of T lymphocytes targeting α-myosin in both healthy individuals and patients with ICI myocarditis. Although it is present in small numbers, it amplifies substantially once activated by signals from the antigen. And the TCR pools of myocardium and skeletal muscle of three patients with ICI myocarditis unexpectedly overlapped with those of α-myosin-specific T cells, which almost certainly allows α-myosin to act as an autoantigen leading to ICI myocarditis.[69,70] α-MyHC is more commonly derived from tumor cells, and The Cancer Genome Atlas (TCGA) melanoma cohort analysis revealed that 250 of 363 tumors had detectable MYH6 expression. Of course, in addition to α-myosin other possible antigens have been reported, such as anti-transverse muscle antibodies and anti-acetylcholine receptors.[71] Remarkably, patients suffering from immune checkpoint inhibitor-induced myocarditis combined with myositis have also exhibited heightened levels of anti-3-hydroxy-3-methylglutaryl coenzyme A reductase antibodies. Nevertheless, to date, only one case has been reported, and the increased levels of anti-3-hydroxy-3-methylglutaryl coenzyme A reductase antibodies were more likely a result of myositis than myocarditis.[57] In general, although the evidence is not clear, almost all studies point in the direction of the idea that tumors express the same antigens as the heart.[69]

In patients with ICI myocarditis, the proportion of T cell subsets is significantly altered. The ratio of Th17 cells is changed, a type of CD4+ cell known for secreting IL-17 and whose clonal expansion leads to the development of inflammation. Th17 cell proliferation is frequently linked to the development of inflammatory conditions such as colitis. Expansion of TH17 cells has been detected in the peripheral blood of some patients with ICI myocarditis, implying that TH17 cells play a role in the development of ICI myocarditis. Why TH 17 cells expand in patients with ICI myocarditis remains a question that needs to be investigated in depth, and the reliable view is that a large amount of TGF-β converts TH1 to TH17 cells with the involvement of IL-6[72] (Figure 2c). Temra cells are a little-studied subset of T cells that are re-expressing CD45+ effector T cells.[73] Effector T cells that re-express CD45RA can secrete a variety of cytokines, including IFN-γ and TNF-α, and exhibit potent cytotoxicity upon activation. CD8+ TEMRA cells characterized by high cytotoxicity accumulate in the elderly and are linked to excessive inflammation. Temra cells are more likely to accumulate in women and older adults, which may explain why older adults and women are more likely to develop ICI myocarditis.[34] A recent single-cell sequencing result showed that Temra cells tend to be highly cloned in patients with ICI-associated myocarditis, and when administered with immunosuppressive agents (glucocorticoid therapy), it shifts to a failing phenotype. Temra cells express elevated levels of a number of cardiac chemokines, including CCL5, CCL4, and CCL4L2, which adds to the conviction that Temra cells play a role in ICI myocarditis.[74] The percentage of Treg cells is also elevated in a subset of patients with ICI myocarditis, but this is not absolute. Klocke et al. found that deletion of CTLA-4 in mice resulted in expansion of Treg cells, which returned to normal levels after six weeks.[75] The mechanism of action is unknown, but it is possible that CTLA-4 regulates CD28-dependent expression of FoxP3 via developing thymocytes. Thus, CTLA-4 deficiency increases the frequency of autoantigen-specific Treg cells in the thymus and periphery. Second, CTLA-4 can inhibit T cell peripheral expansion. When CTLA-4 is blocked or deleted, FoxP3+ Treg cells show greater steady-state expansion than FoxP3 - T cells, resulting in an increase in the number of peripheral FoxP3 + T cells.[76–78] In contrast, PD-L1 regulates the development, maintenance, and function of Treg cells and the anti-PD-1/PD-L1 pathway decreases the proportion of Treg cells.[79] Amarnath et al. suggested that it reduces Th1 cell STAT activation via the phosphatase function of the SHP1/2 signaling pathway located downstream of the PD-1 receptor, thereby reducing the Th1-Treg transition.[80] Of course, these are theoretical, and real-world clinical studies have found that the ratio of Treg cells in patients is not fixed and usually does not change significantly, and we believe that the change in the ratio of Treg cells may be unpredictable due to the different regulatory roles of the CTLA-4 pathway and the PD-1/PD-L1 pathway for Treg cells, but it is clear that regardless of the change in the ratio of Treg cells, Treg cells that are significantly suppressed by ICI are unable to exert their own suppressive effects.[75] By the way, the ICI we mention in this paper are mainly PD-1 and its ligand inhibitors and CTLA-4 inhibitors, mainly because they are approved and widely used ICI. Other ICI also modulate Treg cells, for example, LRBA-deficient patients present with a reduction of Treg cells accompanied by a deficiency of CTLA-4.[81–83]

### Cytokine storm & macrophage infiltration

Cytokine changes directly caused by the PD-1 or CTLA-4 pathways are involved in the development of ICI-myocarditis. Pro-inflammatory cytokines (IFN-γ, TNF-α, IL-17, IL-1, IL-6) are the key factors leading to the occurrence of ICI myocarditis. They damage cardiomyocytes, induce inflammation, and inhibit cardiac repair capacity through multiple pathways.[84,85] The PD-1/PD-L1 pathway inhibits IFN-γ production, and ICI blockade of this pathway leads to a rise in IFN-γ and activation of the inflammatory mediator NFKB/STAT1 pathway, which is a very classical inflammatory pathway that exacerbates autoimmune myocarditis. Of course, in addition to these molecules, which have been mentioned in the majority of articles, new and valuable molecules have also come to our attention. Of course, in addition to these molecules, which have been mentioned in the majority of articles, new and valuable molecules have also come to our attention. For example, angiopoietin-like protein 2 (ANGPTL2), a protein thought to play a role in inflammation in a variety of organs. Haruki Horiguchi suggested that ANGPTL2 could promote the expression of chemokines such as CCL3/4/5 and CXCL10/11 through activation of the NF-KB pathway, which in turn leads to T cell recruitment.[86] NLRP3 and Myd88 are also key players in the cardiotoxicity induced by Nivolumab and Ipilimumab. Vincenzo et al. verified that HFC and mice cultured with anti-PD-L1 and anti-CTLA-4 produced multiple pro-inflammatory cytokines.[57,87] MiRNA is also involved in ICI myocarditis. Exocytosis of ICI-treated macrophages increases MiRNA-34a-5p levels in cardiomyocytes, and MiRNA-34a-5p can target the PNUT gene in cardiomyocytes causing cardiomyocyte senescence[88] (Figure 2b). Long-term use of ICI has the potential to cause cytokine release syndrome (CRS), which is potentially an important cause of death from ICI myocarditis, although CRS mostly occurs with CAR-T therapy.[89] Zhang et al. showed that ICI treatment leads to reduced serum concentrations of 17β-estradiol, especially in female mice, leading to downregulation of MANF and Hspa5 in the heart, and that MANF and HSPA5 are essential for reducing myocardial inflammation in ICI myocarditis. MANF and HSPA5 are transcriptionally enhanced in response to female hormones. Androgens suppress the expression of these genes. This may also explain why women are more prone to ICI myocarditis.[89]

In addition to this, the use of thiazide diuretics may also increase the risk of myocarditis in oncology patients being treated with ICI.[90]

### Direct cardiomyocyte injury & fibrosis

Activated effector T lymphocytes in ICIassociated myocarditis mediate direct cardiomyocyte lysis via the perforin – granzyme B pathway, culminating in necrotic cell death and impaired contractile function; this cytotoxic mechanism has been

validated in CTLA4–deficient murine models.[45] Upregulation of the Fas – Fas ligand apoptotic axis in PD1–deficient mice further exacerbates programmed cardiomyocyte death, amplifying overall myocardial injury.[91] Gasdermin E (GSDME) – mediated pyroptosis is activated in ICIinduced myocarditis: accumulation of cleaved GSDME fragments in cardiomyocytes not only precipitates membrane rupture but also drives release of proinflammatory cytokines, fueling local inflammation.[92] Humoral autoimmunity aggravates tissue damage – MRLPdcd1$^{-/-}$ mice generate high-titer anti – cardiac myosin autoantibodies that associate with marked cardiomyocyte degeneration and interstitial fibrosis.[93] Genetic background modulates the dissociation between inflammation and fibrotic remodeling: PD1 knockout on the BALB/c background results in ventricular dilation, mild ejection fraction decline, and diffuse myocardial degeneration with interstitial fibrosis despite minimal leukocyte infiltration, indicating that loss of PD1 signaling alone suffices to induce cardiomyocyte injury and fibrosis.[94] Persistent cardiomyocyte loss activates cardiac fibroblasts via TGFβ–dependent signaling, promoting their differentiation into myofibroblasts and accelerating collagen and extracellular matrix deposition; experimental blockade of TGFβ markedly attenuates myocardial fibrosis in autoimmune myocarditis models.

## Diagnosis of ICI-associated myocarditis

ICI-related myocarditis is a potentially life-threatening complication of ICI therapy, requiring prompt recognition and a comprehensive diagnostic approach. Clinically, presentations range from asymptomatic elevations in cardiac biomarkers to severe manifestations such as chest pain, arrhythmias, heart failure, or cardiogenic shock. Laboratory testing often reveals elevated troponin I as a marker of myocardial injury, while troponin T may also increase, though with less specificity due to possible concurrent myositis. Natriuretic peptides are frequently elevated, particularly in patients with underlying malignancies. Electrocardiographic abnormalities are common and may include ectopic beats, ST-T changes, or high-grade arrhythmias like complete heart block.[6] Imaging with echocardiography may show regional wall motion abnormalities or preserved systolic function, whereas cardiac magnetic resonance (CMR) offers superior tissue characterization, detecting edema, inflammation, and fibrosis; positron emission tomography may be considered when CMR is contraindicated. Endomyocardial biopsy, though not always feasible, remains the diagnostic gold standard, demonstrating lymphocytic and macrophage infiltration.[95] Differential diagnoses such as acute coronary syndrome or non-ischemic cardiomyopathy must be excluded, often necessitating early coronary angiography. In summary, the diagnosis of ICI-related myocarditis requires a comprehensive approach integrating clinical evaluation, laboratory testing, imaging studies, and, when appropriate, histopathological assessment. Early identification and intervention are critical for improving patient outcomes. Therefore, the discovery of more sensitive and specific biomarkers is essential for enhancing early diagnostic accuracy.

### *Traditional cardiac biomarkers*

Troponin, creatine kinase (CK), CK-MB, B-type natriuretic peptide (BNP), and High-sensitivity troponin are the traditional markers of myocardial injury. In a clinical study, troponin, CK, and CK-MB were found to be significantly different in ICI myocarditis. Patients who died of myocarditis had significantly higher Troponin, CK and CK-MB at presentation than survivors. There was no significant difference in BNP. Troponin is suggestive of the development of ICI-myocarditis, with 94% of patients having elevated troponin at the time of clinical presentation. It is the most reliable and early predictor of progression to severe myocarditis.[96–102] Weekly monitoring of troponin is relevant for the early detection of ICI myocarditis. Early and aggressive cortisol treatment is a key factor in improving survival in grade III and IV ICI-myocarditis.[103,104] In addition, hs-cTnT is also considered to be important in the detection of ICI myocarditis, however, the possibility that myositis leads to elevated hs-cTnT needs to be considered, ICI myocarditis and myositis may coexist because they share antigens.[105–109] Troponin is an inexpensive and easily measurable biomarker that is of great interest in the diagnosis and prognosis of ICI myocarditis. However, it can also be measured in patients with acute coronary syndrome, so the use of these traditional myocardial markers to diagnose ICI myocarditis requires a full assessment of the patient's cardiovascular condition.

### *Inflammatory & immunological biomarkers*

NLR and CRP are very classical markers of inflammation. NLR is defined as absolute neutrophil count/absolute lymphocyte count; CRP, also known as C-reactive protein, is an acute phase protein synthesized by hepatocytes. It is commonly utilized as a biomarker for assessing the presence and severity of inflammation due to its propensity to become elevated in response to inflammatory stimuli.[110] Both indicators were significantly elevated in non-small cell lung cancer Patients who developed irAEs, and the fact that they are easily measured and inexpensive suggests that they may be a promising biomarker.[111] Similar results have been reported in studies of cardiac adverse reactions. Moey et al. indicated that CRP and NLR were significantly higher in patients who experienced MACE compared to their baseline values. But this difference was not only seen in ICI myocarditis, but also in noncardiac irAE.[27,112] Elevated NLR and CRP were also seen in immune-associated pneumonia.[113,114] Incidentally, NLR and CRP are associated with the prognosis of ICI treatment, patients with early CRP reduction and early NLR reduction have longer PFS and OS.[114]

Cytokines are soluble proteins of about 5 to 20 kda in size that can modulate the immune system. ICI myocarditis is an immune injury mediated by T cell, and it is clear that cytokines must be deeply involved in this process. Lim et al. demonstrated that 11 cytokines (G-CSF, GM-CSF, Fractalkine, FGF-2, IFNα2, IL12p70, IL1a, IL1B, IL1RA, IL2, and IL13) were significantly upregulated in patients with melanoma who developed significantly upregulated in melanoma patients with severe irAE.[115] Tsuruda et al. reported three clinical

cases of ICI myocarditis in which IL-8 and G-CSF were the main cytokine elevations observed, but only one of the three patients had myocarditis confirmed by endomyocardial biopsy, and the sample size was too small to make the veracity of this finding questionable.[116] IFN-γ and TNF-α have been confirmed to rise in several studies.[57] Suppressor of tumorigenicity 2 (ST2) is a member of the interleukin 1 (IL-1) receptor/Toll-like superfamily involved in inflammatory processes and the progression of immune diseases.ST2 has four isoforms: two of them are mainly the membrane-bound receptor form (ST2L) and the soluble form (sST2).[117] Wang et al. demonstrated that sST2 is a powerful biomarker for fulminant myocarditis with high specificity and sensitivity, and it even showed better diagnostic performance than cTnI or NT-proBNP, but it has only been investigated in patients with fulminant myocarditis, and further studies are needed to determine whether it can be used for the diagnosis and prediction of ICI myocarditis.[118] In general, there are very few studies on cytokines in studies related to ICI myocarditis, which may be due to the very low prevalence of ICI myocarditis resulting in few clinical samples, and subsequent large-scale multicenter studies are required to identify biomarkers that can predict and diagnose ICI myocarditis.

We previously detailed the mechanistic changes in numerous T cell subsets, including Th17 cells, Th1 cells, Treg cells, and Temra cells. It is obvious that these modifications are likely to indicate the development of ICI myocarditis, but whether they can be employed as a reliable biomarker requires additional exploration. To begin with, Th17 cell amplification is not limited to ICI myocarditis; it occurs in the majority of chronic inflammatory disorders, particularly chronic enteritis, and it is difficult to isolate Th17 cells. Treg cells are unknown in patients with ICI myocarditis, maybe because the type of ICI medication administered differs from patient to patient. As previously mentioned, the PD-1/PD-L1 pathway and the CTLA-4 pathway may have opposing effects on the regulation of Treg cells, and the proportion of Treg cells are not reliable biomarkers at present. Temra cell is an attractive subpopulation for the detection of ICI myocarditis, which is potentially a high-quality biomarker for the detection of ICI myocarditis.

## Emerging biomarkers

MicroRNAs (miRNAs) are short non-coding RNAs that are stable in body tissues and various body fluids and regulate gene expression by targeting mRNAs.[119] These properties suggest that miRNAs are well suited to be a new disease-specific biomarker. miRNAs have been a hot topic in tumor research in recent years, and there are many studies on immune checkpoints and miRNAs. miR-200/ZEB axis is closely associated with high PD-L1 expression.[120,121] miR-197-5p was negatively associated with PD-L1 expression via CKS1B/STAT3 axis.[122] miR-33a-5p was associated with reduced PD-1, PD-L1, and CTLA-4 expression, demonstrating miRNA have predictive potential in ICI-related adverse effects.[123] Several miRNAs, including miR-21a-5p, miR-147-3p, and miR-146b-5p, were upregulated in mice with experimental autoimmune myocarditis, and silencing miR-21a-5p attenuated myocarditis symptoms.[124] Lewandowski et al. showed that miR-Chr8:96, miR-155, and miR-206 were promising biomarkers for myocarditis, and serum levels of the above three miRNAs were positively correlated with the diagnosis of myocarditis and inflammatory dilated cardiomyopathy with high specificity.[125] hsa-miR-Chr8:96 possesses the ability to discriminate myocarditis from myocardial infarction compared with other MiRNA, which can greatly improve the accuracy of myocarditis diagnosis.[99] The expression of miR-21-5p and miR-1-3p was elevated in peripheral blood of patients with acute viral myocarditis, and the elevation correlated with myocardial injury.[126] The exosome miRNA-34a-5p has been identified to play a role in ICI myocarditis, but more research is needed to determine whether the above miRNA can be used in the diagnosis and prediction of ICI myocarditis.[88]

In patients with suspected myocarditis, endomyocardial biopsy (EMB) is the gold standard; however, this invasive test is not applicable to all patients, so cardiovascular magnetic resonance (CMB) and cardiac ultrasound are usually used more frequently to determine cardiac function, and CMB is considered the gold standard for noninvasive tests.[95] Left ventricular ejection fraction (LVEF) and overall longitudinal strain (GLS) are routinely used measurements of cardiac function and cardiac injury, with LVEF < 55% indicating compromised cardiac function.[127,128] However, only 51% of ICI myocarditis actually has a significant decrease in LVEF, which is different from acute myocarditis, so LVEF is not used for the diagnosis of ICI myocarditis.[6,129,130] This may be due to the rapid onset of myocarditis in ICI, where LVEF does not appear significantly altered. GLS was much more sensitive than LVEF in ICI myocarditis, with GLS decreasing with the development of ICI-associated myocarditis and not changing in controls without myocarditis, and lower GLS was a robust predictor of MACE in cases of myocarditis. Higher admission troponin and NT-proBNP levels were linked with decreased GLS in patients, and the degree of GLS reduction was likewise prognostic, with a 1.5-fold increase in MACE for every 1% reduction in cases with reduced EF and a 4.4-fold increase in those with maintained EF.[96]

**Table 2.** ICI myocarditis-related biomarkers.

| Biomarker Category | Example Marker | Sensitivity | Specificity | Advantages | Limitations |
|---|---|---|---|---|---|
| Traditional | cTnI | High (~94%) | Moderate | Early elevation, easy to measure | Affected by ACS, nonspecific |
| | hs-cTnT | Very high | Lower | Extremely sensitive | Influenced by myositis, less specific |
| | CK/CK-MB | Moderate | Low | Reflects myocardial necrosis | Cannot distinguish tumor myopathy |
| Inflammatory | NLR/CRP | Moderate | Low | Widely available, low cost | Lacks cardiac specificity |
| Immunological | sST2 | Moderate to high | Moderate to high | Correlates with myocarditis activity | Limited studies, small sample sizes |
| Molecular | miR-155, miR-Chr8:96 | To be determined | To be determined | High tissue specificity potential | No large-scale clinical validation |

## Future directions

The comparative insights gleaned from evaluation provide a clear framework for selecting appropriate biomarkers at different diagnostic stages (Table 2). Due to the inherent limitations of individual biomarkers, it is often challenging to simultaneously achieve both high sensitivity and specificity. Therefore, future diagnostic workflows for ICI-related myocarditis should aim to establish a seamless integration of multiple biomarkers across three key stages: screening, differential diagnosis, and definitive confirmation.

In the screening phase, routine monitoring of traditional cardiac injury markers such as cTnI and NT-proBNP enables the early identification of patients at risk, with hs-cTnT providing additional sensitivity for early detection. In the differential diagnosis phase, the incorporation of inflammatory and immune-related biomarkers – including NLR, CRP, and soluble ST2 (sST2) – may aid in distinguishing myocarditis from nonspecific systemic inflammation or other immune-related adverse events (irAEs). In the confirmation phase, in cases where imaging findings such as cardiac magnetic resonance (CMR) suggest myocarditis, specificity can be further enhanced by molecular biomarker panels, such as miR-155 and miR-Chr8:96, along with T cell subset profiling. When necessary, endomyocardial biopsy (EMB) remains the gold standard for histopathological confirmation.

To advance the clinical implementation of this multibiomarker approach, large-scale, multicenter prospective cohort studies are warranted. These datasets can be leveraged to develop machine learning – based predictive models that integrate demographic information, biomarker profiles, and imaging parameters. Such models offer promise for real-time risk monitoring and precise stratification of ICI-associated myocarditis. This optimized framework has the potential to significantly improve diagnostic accuracy while reducing the need for invasive procedures. Nevertheless, continued validation and iterative refinement in large clinical settings remain essential.

## Discussion

ICIassociated myocarditis affects approximately 1.14% of patients treated with ICI and typically presents early – within a median of 27–34 days after therapy initiation (interquartile range, 21–75 days).[6,131] Despite its rarity, the condition carries a high case fatality rate of 50%.[37] Pathologically, myocarditis arises from disruption of PD1/PDL1 and CTLA4 checkpoints, leading to T cell – mediated cardiomyocyte injury, cytokine-driven inflammation, macrophage infiltration, direct cytotoxicity via perforin – granzyme and Fas – FasL pathways, autoantibody formation, and subsequent fibrotic remodeling[6] (Figure 2). A variety of biomarkers have been investigated: cardiac troponins (cTnI, hscTnT) and natriuretic peptides show high sensitivity for early detection but lack specificity, inflammatory markers (NLR, CRP) correlate with prognosis yet fail to distinguish myocarditis from other irAEs, while emerging marker such as miR155, miRChr8:96, and sST2 demonstrate promising tissue specificity but remain confined to small, single center or case series studies without large-scale validation.[38,132]

Current guidelines underscore the need for systematic cardiac monitoring in patients receiving ICI. The 2022 ESC CardioOncology Guidelines recommend baseline and predose (cycles 2–4) assessments of ECG and high sensitivity troponin, with transthoracic echocardiography for high-risk individuals, and multidisciplinary management involving cardiology and oncology teams.[133] Similarly, the ESMO Clinical Practice Guidelines advise early recognition of cardiotoxicity, prompt corticosteroid therapy for suspected or confirmed myocarditis, and individualized decisions regarding ICI interruption or rechallenge.[134] Integrating these recommendations with emerging biomarker data may refine surveillance protocols and enable earlier intervention.

There is an urgent need for prospective, multicenter cohort studies to validate candidate biomarkers – particularly miRNAs and sST2—and to define optimal monitoring intervals. Machine learning models that integrate demographic, clinical, imaging (e.g., global longitudinal strain), and molecular marker data could provide realtime risk stratification tools. Mechanistic studies should further elucidate antigen cross-reactivity (e.g., αmyosin epitopes) and the role of preexisting cardiovascular comorbidities in predisposition to myocarditis.[135] Randomized trials comparing steroid regimens and adjunctive immunosuppressants (e.g., abatacept, tocilizumab) are also necessary to balance myocarditis control against anticancer efficacy.

The heterogeneity of available studies – ranging from case reports and small single-center cohorts to pharmacovigilance databases – limits the generalizability of findings. The majority of biomarker investigations lack large-scale, prospective validation, and assay standardization remains a barrier to clinical adoption. Rapid evolution of ICI regimens and combination therapies further complicates the extrapolation of historical data. ICIassociated myocarditis poses a critical challenge in the era of cancer immunotherapy, demanding harmonized, evidence-based diagnostic and management strategies. By integrating guideline recommended monitoring with a multibiomarker approach, there is potential to improve early detection and patient outcomes. Continued collaboration between cardiooncology researchers and clinical consortia will be essential to translate these insights into practice and safeguard cardiovascular health without compromising the transformative benefits of ICI.

## Conclusion

ICI-associated myocarditis is highly lethal, with a median onset time of 34 days and a mortality rate of 50%, underscoring its critical nature. The underlying pathogenesis involves multifactorial immune-mediated injury triggered by blockade of the PD1/PDL1 and CTLA4 pathways, including T cell-mediated cardiotoxicity, cytokine storm, macrophage infiltration, and direct cytotoxic effects. To facilitate early detection, current cardio-oncology guidelines recommend high-sensitivity cardiac troponin and electrocardiogram monitoring before ICI initiation and prior to cycles 2–4, along with echocardiographic evaluation for high-risk patients. Although numerous

traditional (e.g., cTnI, hscTnT, BNP) and emerging biomarkers (e.g., miRNAs, sST2) have been proposed, most studies remain small-scale and retrospective, lacking large prospective multi-center validation. Their diagnostic sensitivity and specificity vary significantly across cohorts, limiting clinical applicability. While myocardial injury markers such as cTnI and CK-MB are frequently elevated in patients with ICI-associated myocarditis, their limited specificity precludes differentiation from other forms of myocardial injury, such as those due to infection or ischemic heart disease. Similarly, inflammatory markers like CRP and IL-6 are commonly elevated in a range of inflammatory or immune conditions and therefore cannot serve as standalone diagnostic indicators for ICI-associated myocarditis. Future research should focus on standardizing detection methods and conducting prospective validation studies. Integrating machine learning models to combine demographic, clinical, molecular, and imaging data may enable real-time risk stratification and personalized management. The main limitation of this review lies in the reliance on case reports and single-center studies, with small sample sizes, methodological heterogeneity, and inconsistent testing protocols. Furthermore, current data do not fully reflect evolving treatment paradigms, which may limit the generalizability of findings. In summary, by synthesizing current cardio-oncology guidelines and biomarker research, this review provides a theoretical framework for the early diagnosis, risk assessment, and individualized treatment of ICI-associated myocarditis, and outlines directions for future large-scale validation and clinical implementation.

## Funding

This work was supported by National Natural Science Foundation of China [No. 31971166].

## ORCID

Ximing Xu http://orcid.org/0000-0002-4240-5378

## Notes on contributor

*Ximing Xu* Standing Committee of Tumor Radiation Protection Specialized Committee of Chinese Anti-cancer Association, Standing Committee of Tumor Thermal Infusion Chemotherapy Group of Chinese Anti-cancer Association, Standing Committee of Tumor Specialized Committee of Chinese Physicians Association of Integrative Medicine, Vice Chairman of Precision Medicine and Tumor MDT Specialized Committee of Chinese Society of Research Hospitals, Member of Tumor Precision Radiotherapy Specialized Committee of Wu Jieping Foundation, Chairman of Tumor Specialized Committee of Hubei Microcirculation Society, Deputy Chairman of Tumor Molecular Targeted Therapy Specialized Committee of Hubei Anti-Cancer Association. Vice Chairman of Tumor Molecular Targeted Therapy Committee of Hubei Anti-cancer Association, Standing Committee of Internal Medicine Treatment Committee of Hubei Anti-cancer Association. He has presided over two National Natural Science Foundation of China, one sub-project of National Key Research and Development Program of China, many projects of Doctoral Fund of Ministry of Education, Natural Science Foundation of Hubei Province, and Wuhan Morning Glory Program. He has published more than 40 SCI papers as the first and the corresponding authors, and dozens of papers in Chinese core journals, and he has edited two monographs, and has won one award of Provincial Scientific and Technological Progress Award.

## Author contributions

LX and YC carried out the primary literature search, drafted and revised the manuscript. Xiong Lin and SW helped modify the manuscript. YS and ZZ involved in the conception and design. XX provided the direction and ideas of writing and made repeated revisions and guidance in the whole process of writing. All authors contributed to the article and approved the submitted version.

## Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

## Ethics statement

This article is a review and does not involve human subjects. Therefore, the ethical statement does not apply to this article.

## References

1. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. Science. 2018;359(6382):1350–1355. doi: 10.1126/science.aar4060.
2. Shiravand Y, Khodadadi F, Kashani SMA, Hosseini-Fard SR, Hosseini S, Sadeghirad H, Ladwa R, O'Byrne K, Kulasinghe A. Immune checkpoint inhibitors in cancer therapy. Current Oncology. 2022;29(5):3044–3060. doi: 10.3390/curroncol29050247.
3. Nüssing S, Trapani JA, Parish IA. Revisiting T cell tolerance as a checkpoint target for cancer immunotherapy. Front Immunol. 2020;11:589641. doi: 10.3389/fimmu.2020.589641.
4. Zimmer L, Goldinger SM, Hofmann L, Loquai C, Ugurel S, Thomas I, Schmidgen MI, Gutzmer R, Utikal JS, Göppner D, et al. Neurological, respiratory, musculoskeletal, cardiac and ocular side-effects of anti-PD-1 therapy. Eur J Cancer. 2016;60:210–225. doi: 10.1016/j.ejca.2016.02.024.
5. Wang DY, Salem J-E, Cohen JV, Chandra S, Menzer C, Ye F, Zhao S, Das S, Beckermann KE, Ha L, et al. Fatal toxic effects associated with immune checkpoint inhibitors: a systematic review and meta-analysis. JAMA Oncol. 2018;4(12):1721–1728. doi: 10.1001/jamaoncol.2018.3923.
6. Mahmood SS, Fradley MG, Cohen JV, Nohria A, Reynolds KL, Heinzerling LM, Sullivan RJ, Damrongwatanasuk R, Chen CL, Gupta D, et al. Myocarditis in patients treated with immune checkpoint inhibitors. J Am Coll Cardiol. 2018;71(16):1755–1764. doi: 10.1016/j.jacc.2018.02.037.
7. Yi J, Kawabe T, Sprent J. New insights on T-cell self-tolerance. Curr Opin Immunol. 2020;63:14–20. doi: 10.1016/j.coi.2019.10.002.
8. Rudd CE, Schneider H. Unifying concepts in CD28, ICOS and CTLA4 co-receptor signalling. Nat Rev Immunol. 2003;3(7):544–556. doi: 10.1038/nri1131.
9. Linsley PS, Brady W, Urnes M, Grosmaire LS, Damle NK, Ledbetter JA. CTLA-4 is a second receptor for the B cell activation antigen B7. J Exp Med. 1991;174(3):561–569. doi: 10.1084/jem.174.3.561.
10. Rudd CE, Taylor A, Schneider H. CD28 and CTLA-4 coreceptor expression and signal transduction. Immunological Rev. 2009;229(1):12–26. doi: 10.1111/j.1600-065X.2009.00770.x.
11. Qureshi OS, Zheng Y, Nakamura K, Attridge K, Manzotti C, Schmidt EM, Baker J, Jeffery LE, Kaur S, Briggs Z, et al. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. Science. 2011;332(6029):600–603. doi: 10.1126/science.1202947.
12. Corse E, Allison JP. Cutting edge: CTLA-4 on effector T cells inhibits in trans. The J Immunol. 2012;189(3):1123–1127. doi: 10.4049/jimmunol.1200695.

13. Tivol EA, Borriello, F, Schweitzer, AN, Lynch, WP, Bluestone, JA, Sharpe, AH. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity. 1995;3(5):541–547. doi: 10.1016/1074-7613(95)90125-6.
14. Ribas A, Hu-Lieskovan S. What does PD-L1 positive or negative mean? J Exp Med. 2016;213(13):2835–2840. doi: 10.1084/jem.20161462.
15. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, Fitz LJ, Malenkovich N, Okazaki T, Byrne MC, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med. 2000;192(7):1027–1034. doi: 10.1084/jem.192.7.1027.
16. Zhong X, Tumang JR, Gao W, Bai C, Rothstein TL. PD-L2 expression extends beyond dendritic cells/macrophages to B1 cells enriched for V H 11/V H 12 and phosphatidylcholine binding. Eur J Immunol. 2007;37(9):2405–2410. doi: 10.1002/eji.200737461.
17. Sharpe AH, Pauken KE. The diverse functions of the PD1 inhibitory pathway. Nat Rev Immunol. 2018;18(3):153–167. doi: 10.1038/nri.2017.108.
18. Grabie N, Gotsman, I, DaCosta, R, Pang, H, Stavrakis, G, Butte, MJ, Keir, ME, Freeman, GJ, Sharpe, AH, Lichtman, AH. Endothelial programmed death-1 ligand 1 (PD-L1) regulates CD8 + T-Cell–Mediated injury in the heart. Circulation. 2007;116 (18):2062–2071. doi: 10.1161/CIRCULATIONAHA.107.709360.
19. Keir ME, Liang SC, Guleria I, Latchman YE, Qipo A, Albacker LA, Koulmanda M, Freeman GJ, Sayegh MH, Sharpe AH, et al. Tissue expression of PD-L1 mediates peripheral T cell tolerance. J Exp Med. 2006;203(4):883–895. doi: 10.1084/jem.20051776.
20. Farrag M, Ibrahim E, Abdelwahab H, Elsergany A, Elhadidy T. PDL-1 expression in lung carcinoma and its correlation with clinicopathological and prognostic characteristics. J Immunoassay Immunochem. 2021;42(6):679–690. doi: 10.1080/15321819.2021.1938606.
21. Hsieh CC, Hsu HS, Li AF, Chen YJ. Clinical relevance of PD-L1 and PD-L2 overexpression in patients with esophageal squamous cell carcinoma. J Thorac Dis. 2018;10(7):4433–4444. doi: 10.21037/jtd.2018.06.167.
22. Eggermont AMM, Kicinski M, Blank CU, Mandala M, Long GV, Atkinson V, Dalle S, Haydon A, Khattak A, Carlino MS, et al. Association between immune-related adverse events and recurrence-free survival among patients with stage III melanoma randomized to receive pembrolizumab or placebo: a secondary analysis of a randomized clinical trial. JAMA Oncol. 2020;6 (4):519–527. doi: 10.1001/jamaoncol.2019.5570.
23. Koelzer VH, Rothschild SI, Zihler D, Wicki A, Willi B, Willi N, Voegeli M, Cathomas G, Zippelius A, Mertz KD. Systemic inflammation in a melanoma patient treated with immune checkpoint inhibitors—an autopsy study. J For Immunother Of Cancer. 2016;4(1):13. doi: 10.1186/s40425-016-0117-1.
24. Champion SN, Stone JR. Immune checkpoint inhibitor associated myocarditis occurs in both high-grade and low-grade forms. Mod Pathol: Off J U States Can Acad Pathol, Inc. 2020;33(1):99–108. doi: 10.1038/s41379-019-0363-0.
25. Makunts T, Saunders IM, Cohen IV, Li M, Moumedjian T, Issa MA, Burkhart K, Lee P, Patel SP, Abagyan R, et al. Myocarditis occurrence with cancer immunotherapy across indications in clinical trial and post-marketing data. Sci Rep. 2021;11 (1):17324. doi: 10.1038/s41598-021-96467-5.
26. Li C, Bhatti SA, Ying J. Immune checkpoint inhibitors—associated Cardiotoxicity. Cancers (Basel). 2022;14(5):1145. doi: 10.3390/cancers14051145.
27. Moey MYY, Tomdio AN, McCallen JD, Vaughan LM, O'Brien K, Naqash AR, Cherry C, Walker PR, Carabello BA. Characterization of immune checkpoint inhibitor-related cardiotoxicity in lung cancer patients from a rural setting. JACC cardiooncol. 2020;2 (3):491–502. doi: 10.1016/j.jaccao.2020.07.005.
28. Rubio-Infante N, Ramírez-Flores YA, Castillo EC, Lozano O, García-Rivas G, Torre-Amione G. Cardiotoxicity associated with immune checkpoint inhibitor therapy: a meta-analysis. Eur J Heart Fail. 2021;23(10):1739–1747. doi: 10.1002/ejhf.2289.
29. Iwasaki S, Hidaka H, Uojima H, Hashimura M, Nabeta T, Sanoyama I, Wada N, Kubota K, Nakazawa T, Shibuya A, et al. A case of immune checkpoint inhibitor-associated myocarditis after initiation of atezolizumab plus bevacizumab therapy for advanced hepatocellular carcinoma. Clin J Gastroenterol. 2021;14 (4):1233–1239. doi: 10.1007/s12328-021-01442-2.
30. Xie X, Wang L, Li Y, Xu Y, Wu J, Lin X, Lin W, Mai Q, Chen Z, Zhang J. Multi-organ immune-related adverse event is a risk factor of immune checkpoint inhibitor-associated myocarditis in cancer patients: a multi-center study. Front Immunol. 2022;13:879900. doi: 10.3389/fimmu.2022.879900.
31. Portolés Hernández A, Blanco Clemente M, Escribano Garcioa D, Velasco Calvo R, Nunez Garcia B, Oteo Dominguez JF, Salas Anton C, Mendez Garcia M, Segovia Cubero J, Dominguez F, et al. Checkpoint inhibitor-induced fulminant myocarditis, complete atrioventricular block and myasthenia gravis—a case report. Cardiovasc Diagn Ther. 2021;11(4):1013–1019. doi: 10.21037/cdt-21-147.
32. Ma R, Wang Q, Meng D, Li K, Zhang Y. Immune checkpoint inhibitors-related myocarditis in patients with cancer: an analysis of international spontaneous reporting systems. BMC Cancer. 2021;21(1):38. doi: 10.1186/s12885-020-07741-0.
33. Zamami Y, Niimura T, Okada N, Koyama T, Fukushima K, Izawa-Ishizawa Y, Ishizawa K. Factors associated with immune checkpoint inhibitor–related myocarditis. JAMA Oncol. 2019;5 (11):1635–1637. doi: 10.1001/jamaoncol.2019.3113.
34. Salumets A, Tserel L, Rumm AP, Türk L, Kingo K, Saks K, Oras A, Uibo R, Tamm R, Peterson H, et al. Epigenetic quantification of immunosenescent CD8 + TEMRA cells in human blood. Aging Cell. 2022;21(5):e13607. doi: 10.1111/acel.13607.
35. Valpione S, Pasquali S, Campana LG, Piccin L, Mocellin S, Pigozzo J, Chiarion-Sileni V. Sex and interleukin-6 are prognostic factors for autoimmune toxicity following treatment with anti-CTLA4 blockade. J Transl Med. 2018;16(1):94. doi: 10.1186/s12967-018-1467-x.
36. Younis A, Mulla W, Matetzky S, Masalha E, Afel Y, Fardman A, Goitein O, Arad M, Mazin I, Beigel R, et al. Sex-based differences in characteristics and In-Hospital outcomes among patients with diagnosed acute myocarditis. Am J Cardiol. 2020;125 (11):1694–1699. doi: 10.1016/j.amjcard.2020.02.040.
37. Zotova L. Immune checkpoint inhibitors-related myocarditis: a review of reported clinical cases. Diagn (Basel). 2023;13 (7):1243. doi: 10.3390/diagnostics13071243.
38. Jensen G, Wang X, Kuempel J, Palaskas N, Chen Z, Yu W, Chen Y, Mohammad H, Luo W, Chang J, et al. Immune checkpoint inhibitor-associated myocarditis: a historical and comprehensive review. Am J Physiol Heart Circ Physiol. 2025;328(4):H734–h751. doi: 10.1152/ajpheart.00687.2024.
39. Muğlu H, Sünger E, Köylü B, Tunalı D, Erol C, Selcukbiricik F, Bilici A, Olmez OF. Late-onset myocarditis following immune checkpoint inhibitors therapy: a case series with literature review. Medicina (Kaunas). 2025;61(2):302. doi: 10.3390/medicina61020302.
40. Panchal V, Momin E, Jain S, Singh A, Ghuman G, Brar V. Immune checkpoint inhibitor-induced myocarditis: a case report of complete heart block and challenges in a patient on pembrolizumab. J Innov Card Rhythm Manag. 2025;16(3):6215–6219. doi: 10.19102/icrm.2025.16033.
41. Salem JE, Manouchehri A, Moey M, Lebrun-Vignes B, Bastarache L, Pariente A, Gobert A, Spano J-P, Balko JM, Bonaca MP, et al. Cardiovascular toxicities associated with immune checkpoint inhibitors: an observational, retrospective, pharmacovigilance study. Lancet Oncol. 2018;19(12):1579–1589. doi: 10.1016/S1470-2045(18)30608-9.
42. Dearden H, Au L, Wang DY, Zimmer L, Eroglu Z, Smith JL, Cuvietto M, Khoo C, Atkinson V, Lo S, et al. Hyperacute toxicity with combination ipilimumab and anti-PD1 immunotherapy. Eur J Cancer. 2021;153:168–178. doi: 10.1016/j.ejca.2021.04.045.

43. Palaskas NL, King NE, Ostos-Mendoza KC, Ruiz-Jurado A, Ali HJ, Koutroumpakis E, Deswal A, Iliescu C, Durand JB, Karimzad K. Pacing solutions for immune checkpoint myocarditis and associated conduction disorders. JACC Case Rep. 2025;30(8):103187. doi: 10.1016/j.jaccas.2024.103187.
44. Mahmood S, Fradley M, Cohen J, Nohria A, Reynolds KL, Heinzerling LM, Sullivan RJ, Damrongwatanasuk R, Chen CL, Gupta D. Myocarditis in patients treated with immune checkpoint inhibitors. J Am Coll Cardiol. 2018;71(16):1755–1764. doi: 10.1016/j.jacc.2018.02.037.
45. Heinzerling L, Ott PA, Hodi FS, Husain AN, Tajmir-Riahi A, Tawbi H, Pauschinger M, Gajewski TF, Lipson EJ, Luke JJ, et al. Cardiotoxicity associated with CTLA4 and PD1 blocking immunotherapy. J For Immunother Of Cancer. 2016;4(1):50. doi: 10.1186/s40425-016-0152-y.
46. Braghieri L, Gharaibeh A, Nkashama L, Abushouk A, Abushawer O, Mehdizadeh-Shrifi A, Honnekeri B, Calabrese C, Menon V, Funchain P, et al. Long-term cardiovascular outcomes of immune checkpoint inhibitor-related myocarditis: a large single-centre analysis. ESC Heart Fail. 2025;12(2):1237–1245. doi: 10.1002/ehf2.15131.
47. O'Shea MP, Karikalan SA, Yusuf A, Barry T, Habib E, O'Shea J, Killian M, Baqal E, Nayak S, Masson R, et al. Complete heart block is a significant predictor of mortality in immune checkpoint inhibitor myocarditis. Cardio-Oncology. 2023;9(1):34. doi: 10.1186/s40959-023-00185-y.
48. Norwood TG, BC Westbrook, DB Johnson, SH Litovsky, NL Terry, SB McKee, AS Gertler, JJ Moslehi, RM Conry. Smoldering myocarditis following immune checkpoint blockade. J For Immunother Of Cancer. 2017;5(1):91. doi: 10.1186/s40425-017-0296-4.
49. Okazaki T, Tanaka Y, Nishio R, Mitsuiye T, Mizoguchi A, Wang J, Ishida M, Hiai H, Matsumori A, Minato N, et al. Autoantibodies against cardiac troponin I are responsible for dilated cardiomyopathy in PD-1-deficient mice. Nat Med. 2003;9(12):1477–1483. doi: 10.1038/nm955.
50. Wang J, IM Okazaki, Yoshida T, Chikuma S, Kato Y, Nakaki F, Hiai H, Honjo T, Okazaki T. PD-1 deficiency results in the development of fatal myocarditis in MRL mice. Int Immunol. 2010;22(6):443–452. doi: 10.1093/intimm/dxq026.
51. Nishimura H, Okazaki T, Tanaka Y, Nakatani K, Hara M, Matsumori A, Sasayama S, Mizoguchi A, Hiai H, Minato N, et al. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. Science. 2001;291(5502):319–322. doi: 10.1126/science.291.5502.319.
52. Li S, Tajiri K, Murakoshi N, Xu D, Yonebayashi S, Okabe Y, Yuan Z, Feng D, Inoue K, Aonuma K, et al. Programmed death-ligand 2 deficiency exacerbates experimental autoimmune myocarditis in mice. Int J Mol Sci. 2021;22(3):1426. doi: 10.3390/ijms22031426.
53. Tarrio ML, Grabie N, Bu DX, Sharpe AH, Lichtman AH. PD-1 protects against inflammation and myocyte damage in T cell-mediated myocarditis. The J Immunol. 2012;188(10):4876–4884. doi: 10.4049/jimmunol.1200389.
54. Lucas JA, Menke J, Rabacal WA, Schoen FJ, Sharpe AH, Kelley VR. Programmed death ligand 1 regulates a critical checkpoint for autoimmune myocarditis and pneumonitis in MRL mice. The J Immunol. 2008;181(4):2513–2521. doi: 10.4049/jimmunol.181.4.2513.
55. Neu N, Rose NR, Beisel KW, Herskowitz AH, Gurri-Glass GR, Craig SW. Cardiac myosin induces myocarditis in genetically predisposed mice. The J Immunol. 1987;139(11):3630–3636. doi: 10.4049/jimmunol.139.11.3630.
56. Liu SY, Huang WC, Yeh HI, Ko CC, Shieh HR, Hung CL, Chen TY, Chen YJ. Sequential blockade of PD-1 and PD-L1 causes fulminant cardiotoxicity—from case report to mouse Model validation. Cancers (Basel). 2019;11(4):580. doi: 10.3390/cancers11040580.
57. Tay WT, Fang YH, Beh ST, Liu YW, Hsu LW, Yen CJ, Liu PY. Programmed cell death-1: programmed cell death-ligand 1 interaction protects human cardiomyocytes against T-Cell mediated inflammation and apoptosis response in vitro. Int J Mol Sci. 2020;21(7):2399. doi: 10.3390/ijms21072399.
58. Du S, Zhou L, Alexander GS, Park K, Yang L, Wang N, Zaorsky NG, Ma X, Wang Y, Dicker AP, et al. PD-1 modulates radiation-induced cardiac toxicity through cytotoxic T lymphocytes. J Thorac Oncol. 2018;13(4):510–520. doi: 10.1016/j.jtho.2017.12.002.
59. Love VA, Grabie N, Duramad P, Stavrakis G, Sharpe A, Lichtman A. CTLA-4 ablation and interleukin-12–Driven differentiation synergistically augment cardiac pathogenicity of cytotoxic T lymphocytes. Circ Res. 2007;101(3):248–257. doi: 10.1161/CIRCRESAHA.106.147124.
60. Friedline RH, Brown DS, Nguyen H, Kornfeld H, Lee J, Zhang Y, Appleby M, Der SD, Kang J, Chambers CA, et al. CD4+ regulatory T cells require CTLA-4 for the maintenance of systemic tolerance. J Exp Med. 2009;206(2):421–434. doi: 10.1084/jem.20081811.
61. Klein L, Robey EA, Hsieh CS. Central CD4(+) T cell tolerance: deletion versus regulatory T cell differentiation. Nat Rev Immunol. 2019;19(1):7–18. doi: 10.1038/s41577-018-0083-6.
62. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell. 2008;133(5):775–787. doi: 10.1016/j.cell.2008.05.009.
63. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, Nomura T, Sakaguchi S. CTLA-4 control over Foxp3+ regulatory T cell function. Science. 2008;322(5899):271–275. doi: 10.1126/science.1160062.
64. Tekguc M, Wing JB, Osaki M, Long J, Sakaguchi S. Treg-expressed CTLA-4 depletes CD80/CD86 by trogocytosis, releasing free PD-L1 on antigen-presenting cells. Proc Natl Acad Sci USA. 2021;118(30). doi: 10.1073/pnas.2023739118.
65. Johnson DB, Balko JM, Compton ML, Chalkias S, Gorham J, Xu Y, Hicks M, Puzanov I, Alexander MR, Bloomer TL, et al. Fulminant myocarditis with combination immune checkpoint blockade. N Engl J Med. 2016;375(18):1749–1755. doi: 10.1056/NEJMoa1609214.
66. Reuben A, Petaccia de Macedo M, McQuade J, Joon A, Ren Z, Calderone T, Conner B, Wani K, Cooper ZA, Tawbi H, et al. Comparative immunologic characterization of autoimmune giant cell myocarditis with ipilimumab. Oncoimmunology. 2017;6(12):e1361097. doi: 10.1080/2162402X.2017.1361097.
67. Lv H, Havari E, Pinto S, Gottumukkala RVSRK, Cornivelli L, Raddassi K, Matsui T, Rosenzweig A, Bronson RT, Smith R, et al. Impaired thymic tolerance to α-myosin directs autoimmunity to the heart in mice and humans. J Clin Invest. 2011;121(4):1561–1573. doi: 10.1172/JCI44583.
68. Grabie N, Lichtman AH, Padera R. T cell checkpoint regulators in the heart. Cardiovasc Res. 2019;115(5):869–877. doi: 10.1093/cvr/cvz025.
69. Axelrod ML, Meijers WC, Screever EM, Qin J, Carroll MG, Sun X, Tannous E, Zhang Y, Sugiura A, Taylor BC, et al. T cells specific for α-myosin drive immunotherapy-related myocarditis. Nature. 2022;611(7937):818–826. doi: 10.1038/s41586-022-05432-3.
70. Won T, Kalinoski HM, Wood MK, Hughes DM, Jaime CM, Delgado P, Talor MV, Lasrado N, Reddy J, Čiháková D, et al. Cardiac myosin-specific autoimmune T cells contribute to immune-checkpoint-inhibitor-associated myocarditis. Cell Rep. 2022;41(6):111611. doi: 10.1016/j.celrep.2022.111611.
71. Rubio-Infante N, Ramírez-Flores YA, Castillo EC, Lozano O, García-Rivas G, Torre-Amione G. A systematic review of the mechanisms involved in immune checkpoint inhibitors cardiotoxicity and challenges to improve clinical safety. Front Cell Dev Biol. 2022;10:851032. doi: 10.3389/fcell.2022.851032.
72. Jiao S, Subudhi SK, Aparicio A, Ge Z, Guan B, Miura Y, Sharma P. Differences in tumor microenvironment dictate T helper lineage polarization and response to immune checkpoint therapy. Cell. 2019;179(5):1177–1190.e13. doi: 10.1016/j.cell.2019.10.029.
73. Henson SM, Riddell NE, Akbar AN. Properties of end-stage human T cells defined by CD45RA re-expression. Curr Opin Immunol. 2012;24(4):476–481. doi: 10.1016/j.coi.2012.04.001.

74. Zhu H, Galdos FX, Lee D, Waliany S, Huang YV, Ryan J, Dang K, Neal JW, Wakelee HA, Reddy SA, Srinivas S, et al. Identification of pathogenic immune cell subsets associated with checkpoint inhibitor–induced myocarditis. Circulation. 2022;146(4):316–335. doi: 10.1161/CIRCULATIONAHA.121.056730.
75. Klocke K, Sakaguchi S, Holmdahl R, Wing K. Induction of autoimmune disease by deletion of CTLA-4 in mice in adulthood. Proc Natl Acad Sci USA. 2016;113(17):E2383–2392. doi: 10.1073/pnas.1603892113.
76. Verhagen J, Gabryšová L, Minaee S, Sabatos CA, Anderson G, Sharpe AH, Wraith DC. Enhanced selection of FoxP3+ T-regulatory cells protects CTLA-4-deficient mice from CNS autoimmune disease. Proc Natl Acad Sci USA. 2009;106(9):3306–3311. doi: 10.1073/pnas.0803186106.
77. Read S, Greenwald R, Izcue A, Robinson N, Mandelbrot D, Francisco L, Sharpe AH, Powrie F. Blockade of CTLA-4 on CD4+CD25+ regulatory T cells abrogates their function in vivo. The J Immunol. 2006;177(7):4376–4383. doi: 10.4049/jimmunol.177.7.4376.
78. Kataoka H, Greenwald R, Izcue A, Robinson N, Mandelbrot D, Francisco L, Sharpe AH, Powrie F. CD25(+)CD4(+) regulatory T cells exert in vitro suppressive activity independent of CTLA-4. Int Immunol. 2005;17(4):421–427. doi: 10.1093/intimm/dxh221.
79. Francisco LM, Salinas VH, Brown KE, Vanguri VK, Freeman GJ, Kuchroo VK, Sharpe AH. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. J Exp Med. 2009;206(13):3015–3029. doi: 10.1084/jem.20090847.
80. Amarnath S, Mangus CW, Wang JCM, Wei F, He A, Kapoor V, Foley JE, Massey PR, Felizardo TC, Riley JL, et al. The PDL1-PD1 axis converts human TH1 cells into regulatory T cells. Sci Transl Med. 2011;3(111):111ra120. doi: 10.1126/scitranslmed.3003130.
81. Yang L, Xue X, Chen X, Wu J, Yang X, Xu L, Tang X, Wang M, Mao H, Zhao X. Abatacept is effective in Chinese patients with LRBA and CTLA4 deficiency. Genes Dis. 2021;8(5):662–668. doi: 10.1016/j.gendis.2020.03.001.
82. Hou TZ, Verma N, Wanders J, Kennedy A, Soskic B, Janman D, Halliday N, Rowshanravan B, Worth A, Qasim W, et al. Identifying functional defects in patients with immune dysregulation due to LRBA and CTLA-4 mutations. Blood. 2017;129(11):1458–1468. doi: 10.1182/blood-2016-10-745174.
83. Wei SC, Meijers WC, Axelrod ML, Anang NA, Screever EM, Wescott EC, Johnson DB, Whitley E, Lehmann L, Courand PY, Mancuso JJ, et al. A genetic mouse Model recapitulates immune checkpoint inhibitor–associated myocarditis and supports a mechanism-based therapeutic intervention. Cancer Discov. 2021;11(3):614–625. doi: 10.1158/2159-8290.CD-20-0856.
84. Frangogiannis NG. The inflammatory response in myocardial injury, repair, and remodelling. Nat Rev Cardiol. 2014;11(5):255–265. doi: 10.1038/nrcardio.2014.28.
85. Pinal-Fernandez I, Quintana A, Milisenda JC, Casal-Dominguez M, Muñoz-Braceras S, Derfoul A, Torres-Ruiz J, Pak K, Dell'Orso S, Naz F, et al. Transcriptomic profiling reveals distinct subsets of immune checkpoint inhibitor induced myositis. Ann Rheumatic Dis. 2023;82(6):829–836. doi: 10.1136/ard-2022-223792.
86. Horiguchi H, Kadomatsu T, Yamashita T, Yumoto S, Terada K, Sato M, Morinaga J, Miyata K, Oike Y. ANGPTL2 promotes immune checkpoint inhibitor-related murine autoimmune myocarditis. Commun Biol. 2023;6(1):965. doi: 10.1038/s42003-023-05338-4.
87. Quagliariello V, Passariello M, Rea D, Barbieri A, Iovine M, Bonelli A, Caronna A, Botti G, De Lorenzo C, Maurea N, et al. Evidences of CTLA-4 and PD-1 blocking agents-induced cardiotoxicity in cellular and preclinical models. J Personalized Med. 2020;10(4):179. doi: 10.3390/jpm10040179.
88. Xia W, Chen H, Chen D, Ye Y, Xie C, Hou M. PD-1 inhibitor inducing exosomal miR-34a-5p expression mediates the cross talk between cardiomyocyte and macrophage in immune checkpoint inhibitor–related cardiac dysfunction. J For Immunother Of Cancer. 2020;8(2):e001293. doi: 10.1136/jitc-2020-001293.
89. Oda H, Ishihara M, Miyahara Y, Nakamura J, Kozuka Y, Iwasa M, Tsunoda A, Yamashita Y, Saito K, Mizuno T, et al. First case of cytokine release syndrome after nivolumab for gastric cancer. Case Rep Oncol. 2019;12(1):147–156. doi: 10.1159/000496933.
90. Mitsuboshi S, Hamano H, Niimura T, Ozaki AF, Patel PM, Lin T-J, Tanaka Y, Kimura I, Iwata N, Shiromizu S, et al. Association between immune checkpoint inhibitor-induced myocarditis and concomitant use of thiazide diuretics. Int J Cancer. 2023;153(8):1472–1476. doi: 10.1002/ijc.34616.
91. Liu YX, Song YJ, Liu XH, Xu SC, Kong C, Chen LF, Qian H, Wu W. PD-1 inhibitor induces myocarditis by reducing regulatory T cells, activating inflammatory responses, promoting myocardial apoptosis and autophagy. Cytokine. 2022;157:155932. doi: 10.1016/j.cyto.2022.155932.
92. Sun SJ, Jiao X-D, Chen Z-G, Cao Q, Zhu J-H, Shen Q-R, Liu Y, Zhang Z, Xu F-F, Shi Y, et al. Gasdermin-E-mediated pyroptosis drives immune checkpoint inhibitor-associated myocarditis via cGAS-STING activation. Nat Commun. 2024;15(1):6640. doi: 10.1038/s41467-024-50996-5.
93. Buehning F, Lerchner T, Vogel J, Hendgen-Cotta UB, Totzeck M, Rassaf T, Michel L. Preclinical models of cardiotoxicity from immune checkpoint inhibitor therapy. Basic Res Cardiol. 2025;120(1):171–185. doi: 10.1007/s00395-024-01070-0.
94. Čiháková D. T cells and macrophages drive pathogenesis of immune checkpoint inhibitor myocarditis. Circulation. 2024;149(1):67–69. doi: 10.1161/CIRCULATIONAHA.123.067189.
95. Caforio AL, Pankuweit S, Arbustini E, Basso C, Gimeno-Blanes J, Felix SB, Fu M, Helio T, Heymans S, Jahns R, et al. Current state of knowledge on aetiology, diagnosis, management, and therapy of myocarditis: a position statement of the European Society of cardiology working group on myocardial and pericardial diseases. Eur Heart J. 2013;34(33):2636–2648, 2648a–2648d. doi: 10.1093/eurheartj/eht210.
96. Awadalla M, Mahmood SS, Groarke JD, Hassan MZO, Nohria A, Rokicki A, Murphy SP, Mercaldo ND, Zhang L, Zlotoff DA, et al. Global longitudinal strain and cardiac events in patients with immune checkpoint inhibitor-related myocarditis. J Am Coll Cardiol. 2020;75(5):467–478. doi: 10.1016/j.jacc.2019.11.049.
97. Puzanov I, Subramanian P, Yatsynovich YV, Jacobs DM, Chilbert MR, Sharma UC, Ito F, Feuerstein SG, Stefanovic F, Switzer B, et al. Clinical characteristics, time course, treatment and outcomes of patients with immune checkpoint inhibitor-associated myocarditis. J For Immunother Of Cancer. 2021;9(6):e002553. doi: 10.1136/jitc-2021-002553.
98. Touat M, Maisonobe T, Knauss S, Ben Hadj Salem O, Hervier B, Auré K, Szwebel T-A, Kramkimel N, Lethrosne C, Bruch J-F, et al. Immune checkpoint inhibitor-related myositis and myocarditis in patients with cancer. Neurology. 2018;91(10):e985–e994. doi: 10.1212/WNL.0000000000006124.
99. Blanco-Domínguez R, Sánchez-Díaz R, de la Fuente H, Jiménez-Borreguero LJ, Matesanz-Marín A, Relaño M, Jiménez-Alejandre R, Linillos-Pradillo B, Tsilingiri K, Martín-Mariscal ML, et al. A novel circulating MicroRNA for the detection of acute myocarditis. N Engl J Med. 2021;384(21):2014–2027. doi: 10.1056/NEJMoa2003608.
100. Ganatra S, Neilan TG. Immune checkpoint inhibitor-associated myocarditis. Oncologist. 2018;23(8):879–886. doi: 10.1634/theoncologist.2018-0130.
101. Zhuang Y, An Q, Wang F, Han D, Qiao Z, Jiang Q, Liu M, Li Y, Shangguan J, Bi X, Shen D, et al. The role of circulating biomarkers in predicting the 30-day mortality of immune checkpoint inhibitors-related myocarditis: a retrospective cohort study. Intern Emerg Med. 2023;19(2):377–389. doi: 10.1007/s11739-023-03481-8.
102. Xu L, Xu M, Sun W, Zhang W, Song Z. Clinical characteristics and prognostic impact of immune checkpoint inhibitor-associated myocarditis in advanced non-small cell lung cancer. Investigational New Drugs. 2023;41(6):816–824. doi: 10.1007/s10637-023-01400-4.

103. Stefanovic F, Gomez-Caminero A, Jacobs DM, Subramanian P, Puzanov I, Chilbert MR, Feuerstein SG, Yatsynovich Y, Switzer B, Schentag JJ, et al. Neural net modeling of checkpoint inhibitor related myocarditis and steroid response. Clin Pharmacol. 2022;14:69–90. doi: 10.2147/CPAA.S369008.
104. Furukawa A, Tamura Y, Taniguchi H, Kawamura A, Nagase S, Hayashi A, Tada Y, Sase K, Hatake K. Prospective screening for myocarditis in cancer patients treated with immune checkpoint inhibitors. J Cardiol. 2023;81(1):63–67. doi: 10.1016/j.jjcc.2022.07.009.
105. Ruperti-Repilado FJ, van der Stouwe JG, Haaf P, Mueller C, Läubli H, Pfister O, Rothschild SI, Kuster GM, et al. Case report of elevation of high-sensitivity cardiac troponin T in the absence of cardiac involvement in immune checkpoint inhibitor-associated myositis. Eur Heart J Case Rep. 2022;6(9):ytac353. doi: 10.1093/ehjcr/ytac353.
106. Waissengein B, Abu Ata B, Merimsky O, Shamai S, Wolf I, Arnold JH, Bar-On T, Banai S, Khoury S, Laufer-Perl M. The predictive value of high sensitivity troponin measurements in patients treated with immune checkpoint inhibitors. Clin Res Cardiol. 2022;112(3):409–418. doi: 10.1007/s00392-022-02118-8.
107. Lehmann LH, Heckmann MB, Bailly G, Finke D, Procureur A, Power JR, Stein F, Bretagne M, Ederhy S, Fenioux C, et al. Cardiomuscular biomarkers in the diagnosis and prognostication of immune checkpoint inhibitor myocarditis. Circulation. 2023;148(6):473–486. doi: 10.1161/CIRCULATIONAHA.123.062405.
108. Delombaerde D, De Sutter J, Croes L, Vervloet D, Moerman V, Van de Veire N, Willems A-M, Wouters K, Peeters M, Prenen H, et al. Extensive CArdioVAscular characterization and follow-up of patients receiving immune checkpoint inhibitors: a prospective multicenter study. Pharmaceuticals (Basel). 2023;16(4):625. doi: 10.3390/ph16040625.
109. Waissengein B, Abu Ata B, Merimsky O, Shamai S, Wolf I, Arnold JH, Bar-On T, Banai S, Khoury S, Laufer-Perl M, et al. The predictive value of high sensitivity troponin measurements in patients treated with immune checkpoint inhibitors. Clin Res Cardiol. 2023;112(3):409–418. doi: 10.1007/s00392-022-02118-8.
110. Eklund CM. Proinflammatory cytokines in CRP baseline regulation. Adv Clin Chem. 2009;48:111–136.
111. Pavan A, Calvetti L, Dal Maso A, Attili I, Del Bianco P, Pasello G, Guarneri V, Aprile G, Conte P, Bonanno L, et al. Peripheral blood markers identify risk of immune-related toxicity in advanced non-small cell lung cancer treated with immune-checkpoint inhibitors. Oncologist. 2019;24(8):1128–1136. doi: 10.1634/theoncologist.2018-0563.
112. Drobni ZD, Zafar A, Zubiri L, Zlotoff DA, Alvi RM, Lee C, Hartmann S, Gilman HK, Villani AC, Nohria A, Groarke JD, et al. Decreased absolute lymphocyte count and increased Neutrophil/lymphocyte ratio with immune checkpoint inhibitor–associated myocarditis. J Am Heart Assoc. 2020;9(23):e018306. doi: 10.1161/JAHA.120.018306.
113. Akamine T, Takada K, Toyokawa G, Kinoshita F, Matsubara T, Kozuma Y, Haratake N, Takamori S, Hirai F, Tagawa T, et al. Association of preoperative serum CRP with PD-L1 expression in 508 patients with non-small cell lung cancer: a comprehensive analysis of systemic inflammatory markers. Surg Oncol. 2018;27(1):88–94. doi: 10.1016/j.suronc.2018.01.002.
114. Matsuzawa R, Morise M, Kinoshita F, Tanaka I, Koyama J, Kimura T, Kondoh Y, Tanaka T, Shima K, Hase T, Wakahara K, et al. Non-invasive early prediction of immune checkpoint inhibitor efficacy in non-small-cell lung cancer patients using on-treatment serum CRP and NLR. J Cancer Res Clin Oncol. 2022.
115. Lim SY, Lee JH, Gide TN, Menzies AM, Guminski A, Carlino MS, Breen EJ, Yang JY, Ghazanfar S, Kefford RF, Scolyer RA, et al. Circulating cytokines predict immune-related toxicity in melanoma patients receiving anti-PD-1–Based immunotherapy. Clin Cancer Res. 2019;25(5):1557–1563. doi: 10.1158/1078-0432.CCR-18-2795.
116. Tsuruda T, Yoshikawa N, Kai M, Yamaguchi M, Toida R, Kodama T, Kajihara K, Kawabata T, Nakamura T, Sakata K. The cytokine expression in patients with cardiac complication after immune checkpoint inhibitor therapy. Intern Med. 2021;60(3):423–429. doi: 10.2169/internalmedicine.5317-20.
117. Dudek M, Kałużna-Oleksy M, Migaj J, Straburzyńska-Migaj E. Clinical value of soluble ST2 in cardiology. Adv Clin Exp Med. 2020;29(10):1205–1210. doi: 10.17219/acem/126049.
118. Wang J, He M, Li H, Chen Y, Nie X, Cai Y, Xie R, Li L, Chen P, Sun Y, et al. Soluble ST2 is a sensitive and specific biomarker for fulminant myocarditis. J Am Heart Assoc. 2022;11(7):e024417. doi: 10.1161/JAHA.121.024417.
119. Carthew RW, Sontheimer EJ. Origins and mechanisms of miRnas and siRnas. Cell. 2009;136(4):642–655. doi: 10.1016/j.cell.2009.01.035.
120. Chen L, Gibbons DL, Goswami S, Cortez MA, Ahn Y-H, Byers LA, Zhang X, Yi X, Dwyer D, Lin W, et al. Metastasis is regulated via microRNA-200/ZEB1 axis control of tumour cell PD-L1 expression and intratumoral immunosuppression. Nat Commun. 2014;5(1):5241. doi: 10.1038/ncomms6241.
121. Noman MZ, Janji B, Abdou A, Hasmim M, Terry S, Tan TZ, Mami-Chouaib F, Thiery JP, Chouaib S. The immune checkpoint ligand PD-L1 is upregulated in EMT-activated human breast cancer cells by a mechanism involving ZEB-1 and miR-200. Oncoimmunology. 2017;6(1):e1263412. doi: 10.1080/2162402X.2016.1263412.
122. Fujita Y, Yagishita S, Hagiwara K, Yoshioka Y, Kosaka N, Takeshita F, Fujiwara T, Tsuta K, Nokihara H, Tamura T, et al. The clinical relevance of the miR-197/CKS1B/STAT3-mediated PD-L1 network in chemoresistant non-small-cell lung cancer. Mol Ther: J Am Soc Gene Ther. 2015;23(4):717–727. doi: 10.1038/mt.2015.10.
123. Iqbal MA, Arora S, Prakasam G, Calin GA, Syed MA. MicroRNA in lung cancer: role, mechanisms, pathways and therapeutic relevance. Mol Aspects Med. 2019;70:3–20. doi: 10.1016/j.mam.2018.07.003.
124. Mirna M, Paar V, Topf A, Kraus T, Sotlar K, Aigner A, Ewe A, Watzinger S, Podesser BK, Hackl M, et al. A new player in the game: treatment with antagomiR-21a-5p significantly attenuates histological and echocardiographic effects of experimental autoimmune myocarditis. Cardiovasc Res. 2022;118(2):556–572. doi: 10.1093/cvr/cvab015.
125. Lewandowski P, Goławski M, Baron M, Reichman-Warmusz E, Wojnicz R. A systematic review of miRNA and cfDNA as potential biomarkers for liquid biopsy in myocarditis and inflammatory dilated cardiomyopathy. Biomolecules. 2022;12(10):1476. doi: 10.3390/biom12101476.
126. Marketou M, Kontaraki J, Patrianakos A, Kochiadakis G, Anastasiou I, Fragkiadakis K, Plevritaki A, Papadaki ST, Chlouverakis G, Parthenakis F, et al. Peripheral blood MicroRNAs as potential biomarkers of myocardial damage in acute viral myocarditis. Genes (Basel). 2021;12(3):420. doi: 10.3390/genes12030420.
127. Thavendiranathan P, Poulin F, Lim KD, Plana JC, Woo A, Marwick TH. Use of myocardial strain imaging by echocardiography for the early detection of cardiotoxicity in patients during and after cancer chemotherapy: a systematic review. J Am Coll Cardiol. 2014;63(25):2751–2768. doi: 10.1016/j.jacc.2014.01.073.
128. Potter E, Marwick TH. Assessment of left ventricular function by echocardiography: the case for routinely adding global longitudinal strain to ejection fraction. JACC Cardiovasc Imag. 2018;11(2):260–274. doi: 10.1016/j.jcmg.2017.11.017.
129. Zhang L, Awadalla M, Mahmood SS, Nohria A, Hassan MZO, Thuny F, Zlotoff DA, Murphy SP, Stone JR, Golden DLA, et al. Cardiovascular magnetic resonance in immune checkpoint inhibitor-associated myocarditis. Eur Heart J. 2020;41(18):1733–1743. doi: 10.1093/eurheartj/ehaa051.
130. Tamura Y, Tamura Y. Usefulness of longitudinal strain to assess cancer therapy-related cardiac dysfunction and immune

checkpoint inhibitor-induced myocarditis. Pharmaceuticals (Basel). 2023;16(9):1297. doi: 10.3390/ph16091297.
131. Palaskas N, Lopez-Mattei J, Durand JB, Iliescu C, Deswal A. Immune checkpoint inhibitor myocarditis: pathophysiological characteristics, diagnosis, and treatment. J Am Heart Assoc. 2020;9(2):e013757. doi: 10.1161/JAHA.119.013757.
132. Fan Q, Hu Y, Yang C, Zhao B. Myocarditis following the use of different immune checkpoint inhibitor regimens: a real-world analysis of post-marketing surveillance data. Int Immunopharmacol. 2019;76:105866. doi: 10.1016/j.intimp.2019.105866.
133. Lyon AR, López-Fernández T, Couch LS, Asteggiano R, Aznar MC, Bergler-Klein J, Boriani G, Cardinale D, Cordoba R, Cosyns B, et al. 2022 ESC guidelines on cardio-oncology developed in collaboration with the European Hematology Association (EHA), the European Society for therapeutic radiology and oncology (ESTRO) and the International cardio-oncology Society (IC-OS): developed by the task force on cardio-oncology of the European Society of Cardiology (ESC). Eur Heart J. 2022;43(41):4229–4361. doi: 10.1093/eurheartj/ehac244.
134. Haanen J, Obeid M, Spain L, Carbonnel F, Wang Y, Robert C, Lyon AR, Wick W, Kostine M, Peters S, et al. Management of toxicities from immunotherapy: ESMO clinical practice guideline for diagnosis, treatment and follow-up☆. Ann Oncol. 2022;33(12):1217–1238. doi: 10.1016/j.annonc.2022.10.001.
135. López-Fernández T, Lyon AR, Herrmann J. 2022 ESC guidelines on cardio-oncology: how can we improve the cardiovascular health of patients with cancer and cancer survivors? Eur Heart J Cardiovasc Pharmacother. 2022;9(1):4–5. doi: 10.1093/ehjcvp/pvac051.