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Review Article

Cardiovascular toxicity induced by immune checkpoint inhibitors: mechanisms linking immune dysregulation and myocardial fibrosis

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Abstract: Immune checkpoint inhibitors (ICIs) play a key role in cancer immunotherapy, enhancing outcomes for patients with multiple malignancies by blocking the programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) pathways. Nevertheless, their application is limited due to immune-related adverse events (irAEs), which comprise various manifestations, among which cardiovascular toxicities - primarily myocarditis, arrhythmias, pericarditis, and heart failure - are a major clinical challenge. While relatively rare, myocarditis has a fatality rate as high as 50%. The mechanisms underlying ICI-induced cardiovascular toxicity remain not fully understood, especially regarding how immune dysregulation translates into myocardial fibrosis. Herein, the immune dysregulation-inflammation-fibrosis axis is reviewed, and the mechanisms by which immune checkpoint blockade breaks cardiac immune tolerance, inducing T-cell activation, cytokine storms, and the subsequent production of anti-cardiac antibodies, are discussed. Moreover, the important function of non-coding RNAs (ncRNAs) in immunomodulatory and fibrotic pathways is then revealed as a key player in ICI-associated cardiac injury. It also discusses therapeutic strategies involving immune modulation and anti-fibrotic mechanisms, which may be suitable to reduce cardiovascular toxicity as well as improve the overall prognosis of patients. An improved mechanistic understanding underlying these processes will offer new theoretical insights into how to most effectively utilize ICIs safely.

Keywords: Immune checkpoint inhibitors, cardiovascular toxicity, immune dysregulation, myocardial fibrosis, non-coding RNAs, myocarditis

Introduction

ICIs represent a milestone in the field of cancer immunotherapy. ICIs, which block key signaling pathways such as PD-1/PD-L1 and CTLA-4, have significantly improved the survival rates of patients with many different types of malignant tumors [1]. Cardiovascular toxicity is a prominent irAE associated with ICIs. It can manifest as myocarditis, arrhythmias, pericarditis, and heart failure, with potential for fatal evolution in severe cases. The incidence is relatively low, approximately 1-2% for myocarditis, but the corresponding mortality rate is extremely high, ranging from 25-50%. Often, this toxicity is accompanied by myositis and neuropathy, which leads to a key bottleneck that limits the clinical application of ICIs [2]. In the last few years, with indications for ICIs expanding, a need to further dissect mechanisms of cardiovascular toxicity has developed - particularly to understand the molecular pathways through which immune dysregulation ultimately leads to myocardial fibrosis.

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At the pathological level, ICIs disrupt immune tolerance by blocking the PD-1/PD-L1 or CTLA-4 pathways. This leads to T-cell overactivation, a pro-inflammatory cytokine storm (IL-6, tumor necrosis factor-alpha [TNF-α], interferon-gamma [IFN-γ]), and the generation of anti-myocardial antibodies. Recent studies have shown that immune dysregulation may subsequently drive chronic inflammation. This process is responsible for cardiac fibroblast (CF) activation and extracellular matrix (ECM) deposition. Of all these proposed mechanisms, transforming growth factor-beta (TGF-β)/small mothers against decapentaplegic (Smad) signaling is supported by clinical and translational evidence suggesting its importance in CF activation and ECM deposition, whereas other pathways, such as nuclear factor-kappa B (NF-κB), are mostly based on experimental studies and ultimately contribute to the development of myocardial fibrosis [3, 4]. Nonetheless, these findings mainly derive from in vitro and animal studies, and only a limited subset has been explored clinically [5]. While different ncRNAs have been linked to the regulation of fibrotic pathways, most of these observations have been in vitro or animal studies and only a few have been evaluated clinically. For this reason, their relevance to ICI-induced cardiovascular toxicity remains uncertain [6, 7]. These molecules provide new directions for early diagnosis and targeted intervention.

ICI-related cardiovascular toxicity is heterogeneous: myocarditis shows $CD8^+$ T cell infiltration and myocardial cell apoptosis; by contrast, non-inflammatory left ventricular dysfunction (LVD) lacks typical inflammatory markers but does exhibit fibrotic remodeling, while pericardial disease and vasculitis are associated with the activation of unique immune cell subgroups. Combination ICI therapies, such as combined PD-1 and CTLA-4 inhibitors, are known to significantly increase the risk of myocarditis compared to monotherapy, likely highlighting the dual effects of immune synergism [8].

This review aims to systematically analyze the "immune dysregulation-inflammation-fibrosis" axis of ICI cardiovascular toxicity. It focuses on the following core issues: (1) How immune checkpoint blockade disrupts cardiac immune tolerance and triggers autoimmune responses; (2) How chronic inflammation promotes fibrosis through key signaling pathways (such as TGF-β/Smad, IL-6/Janus kinase [JAK]/signal transducer and activator of transcription 3 [STAT3]); (3) How ncRNAs mediate immune-fibrosis interactions; (4) The translational potential of therapeutic targets (such as immune modulation, anti-fibrosis drugs, and combination strategies). By integrating basic research with clinical evidence, this article will provide a theoretical basis for optimizing the management of ICI-related cardiovascular toxicity and promote precision treatment strategies through interdisciplinary collaboration.

Cardiovascular toxicity induced by ICIs: clinical features and pathophysiology

Clinical features

Immune checkpoint blockade creates a pro-inflammatory phenotype, increases myocardial expression of NOD-like receptor family pyrin domain containing 3 (NLRP3), and thereby contributes to cardiac toxicity associated with combination ICI therapies [9]. Myocarditis is a rare but potentially fatal irAE induced by ICIs [10]. Fatal myocarditis has been reported in patients with renal cell carcinoma during combined anti-PD-L1 and tyrosine kinase inhibitor therapy; this led to the suggestion that PD-1/PD-L1 blockade-mediated immune activation is likely the principal trigger, while the contribution from concomitant TKIs remains uncertain [11, 12]. PD-1/PD-L1 pathway disruption can promote immune-mediated cardiac injury, evidenced by autoimmune cardiomyopathy in PD-1-deficient mice and myocardial T-cell infiltration in non-human primates treated with ICIs; however, whether this is relevant to human ICI-associated cardiotoxicity requires further validation [13, 14]. Patients with myocarditis can present with a heterogeneous clinical course throughout the disease [15]. Approximately half of the patients experience spontaneous remission, while about 25% develop persistent heart failure, and up to 25% may have fatal outcomes or progress rapidly to end-stage heart failure, requiring heart transplantation [16]. Immune checkpoint inhibitor-associated myocarditis (ICIAM) is usually accompanied by myositis and peripheral neuropathy. The most common clinical findings in myocarditis patients are elevated cardiac troponin I (cTnI) levels and electrocardiogram (ECG) abnormalities (including T-wave or ST-segment changes, arrhyth-

mias), which are prognostic indicators [17]. Studies have shown that most ICI-related arrhythmias occur in the context of myocarditis, with a low incidence of new-onset arrhythmias in the first 6 months post-ICI treatment [18]. The risk of arrhythmias increases with wider QRS complexes, prolonged QT intervals, and longer corticosteroid use [19]. PD-1/PD-L1 therapy may induce arrhythmic events, with atrial fibrillation, cardiac arrest, and tachyarrhythmia being the most common adverse arrhythmias [20]. During ICI therapy, ECG markers related to arrhythmia risk do not show significant changes [21].

The use of ICIs is associated with an increased risk of pericardial disease in cancer patients, which is an under-recognized toxicity of ICI therapy. Pericardial effusion in cancer patients treated with PD-1 inhibitors is linked to higher mortality rates [22, 23]. Typical clinical manifestations of ICI-associated pericardial disease include acute chest pain (often exacerbated by changes in body position), pericardial friction rub, and widespread ST-segment elevation on the ECG. Some patients may rapidly progress to cardiac tamponade (manifested by jugular venous distention, pulsus paradoxus, and hypotension) [24]. Cardiac fibrosis caused by the combination of PD-1 inhibitors and chest radiation therapy may be a significant factor influencing long-term survival in patients with thoracic malignancies [25]. ICIs can also lead to a high incidence of LVD, with echocardiography being helpful in early detection of LVD. Baseline hypertension or poor left ventricular systolic or diastolic function are predictive factors for LVD after ICI therapy [26]. Non-inflammatory LVD primarily manifests as asymptomatic reductions in left ventricular ejection fraction (LVEF) ≤ 50% or mild decreases in LVEF (50-53%) accompanied by heart failure symptoms (such as decreased exercise tolerance), but without the typical elevation of inflammatory markers (such as troponin) or evidence of active inflammation on cardiac magnetic resonance (CMR) [27]. ICI-associated vasculitis is a rare early-onset irAE with a severe disease course [28, 29]. Studies have shown that small-vessel vasculitis induced by ipilimumab presents clinically with cyanosis and severe pain in the distal fingers, accompanied by progressive ischemic changes, eventually leading to dry gangrene of the distal digits. Angiography confirms small vessel occlusive lesions, without involvement of larger proximal vessels, which is characteristic of ICI-associated small-vessel vasculitis [30].

Histopathological features

Patients with myocarditis following ICI treatment exhibit changes in immune cell populations, where $CD8^+$ T cells, muscle cell antigens, and inflammatory cytokines are potential key factors contributing to ICIAM. $CD4^+$ central memory T cells (TCM) play a critical role in cardiac protection during ICI therapy. IL-15, IL-4I1, and $CD4^+$ TCM cells may serve as therapeutic targets to reduce ICI-related myocarditis in cancer patients [31]. Low-intensity pulsed ultrasound can improve ICI-induced inflammatory myocardial injury by reducing $CD4^+$ T cell infiltration into the myocardium, inhibiting Th17 differentiation, activating the transcription factor forkhead box P3 (FOXP3), and promoting regulatory T cell (Treg) differentiation [32]. Pericardial T cells are primarily $CD69^+$ tissue-resident memory cells that upregulate PD-1, while pericardial macrophages produce IL-15 to support and maintain tissue-resident memory T cells in the pericardium. Injury-induced myosin-specific tissue-resident memory T cells drive ICI-induced autoimmune myocarditis [12].

Murine CFs have been shown to express high levels of angiopoietin-like protein 2 (ANGPTL2), which might be related to immune modulation but needs clinical confirmation in ICI-related cardiotoxicity [33]. Immunoproteasome proteolysis blockade decreases pro-inflammatory cytokine responses, attenuates the amplification of autoreactive $CD4^+$ T cell expansion, and ameliorates cardiac autoimmune pathology [34]. Studies have demonstrated that targeting the NLRP3 inflammasome can decrease cardiac inflammation in experimental models, but whether it is effective for ICI-related cardiac toxicity has yet to be established in clinical studies [35]. It has also been reported that baricitinib, a representative JAK inhibitor, may be able to modulate macrophage polarization in experimental models, but whether it is protective in ICI-related myocarditis remains to be clinically validated [36]. The SOCS3/JAK/STAT3 signaling pathway, which controls macrophage polarization, is associated with ICI-induced cardiac toxicity due to PD-1/PD-L1 inhibition. After ICI intervention, the levels of $CD86^+$ and MHCII+ are significantly enhanced, with sub-

stantial elevations of macrophage-containing cells [37]. Collagen plays a crucial role in maintaining cardiac structure, elasticity, and signaling transduction. PD-1 inhibition impairs collagen homeostasis via endothelial-fibroblast crosstalk and EndMT, worsening cardiac dysfunction [38]. Gut barrier dysfunction has also been hypothesized to modulate cardiac toxicity via metabolic-inflammatory pathways, although the precise mechanistic role of gut barrier loss in ICI-related cardiotoxicity should be clinically validated [39]. Activation of extracellular signal-regulated kinase 1/2 (ERK1/2) promotes fibroblast proliferation and collagen production experimentally, but its direct contribution to ICI-induced fibrosis has not been clarified clinically [40]. Dendritic cells deficient in 4E-BP3 produce decreased levels of IL-6 and IL-1β, and this functional defect also leads to a diminished capacity for $CD4^+$ T cells to differentiate into Th1 and Th17 cells, which are critical to the pathogenesis of α-myosin-specific T cell-mediated myocarditis [41]. Mechanistically, the CXCR3-CXCL9/10 axis contributes to the recruitment and accumulation of macrophages ($CXCL9^+CCR2^+$) as well as clonally expanded $CD8^+$ T cells (CXCR3hi) in the ICI myocarditis mouse model, but its clinical applicability as a therapeutic target in patients with ICI myocarditis remains to be validated [42]. In experimental models, gasdermin E (GSDME) in cardiomyocytes has been implicated in promoting immune cell infiltration and inflammation; however, its role in human ICI-associated cardiotoxicity remains unclear [43]. In patients with ICI-related myocarditis, there is a significant increase in clonally expanded cytotoxic T effector memory cells re-expressing CD45RA (Temra) $CD8^+$ cells in the blood, which exhibit unique transcriptional changes, including upregulation of chemokines CCL5/CCL4/CCL4L2 [44]. ICI-induced lymphocytic myocarditis is characterized by infiltration of $CD8^+$ T cells and $CD163^+$ tissue macrophages, accompanied by abnormal PD-L1 expression in cardiomyocytes and muscle cell damage [45].

Initiation of immune dysregulation in ICI-induced cardiovascular toxicity

Disruption of immune tolerance

PD-1/PD-L1 and CTLA-4 are key inhibitory checkpoint pathways that restrain excessive T-cell activation and help maintain cardiac immune homeostasis. Blockade of these pathways, particularly PD-1/PD-L1 inhibition, may reactivate cardiac antigen-specific autoreactive T cells, enhance pro-inflammatory cytokine expression, and lower the threshold for autoimmune myocardial injury [46]. Dysregulation of this pathway can lead to pathological cardiac immune damage, such as myocarditis and transplant rejection [47].

ICIs inhibit cardiac immune tolerance by blocking the PD-1/PD-L1 and CTLA-4 pathways, leading to T-cell overactivation and attack on myocardial cells. Experimental evidence indicates that combined CTLA-4 and PD-1 blockade can activate innate inflammatory signaling in myocardial tissue and amplify $CD8^+$ T-cell-mediated injury, linking checkpoint inhibition to early immune-mediated cardiotoxicity [48]. PD-L1 accumulates in cardiac tissue to maintain self-tolerance, and disruption of the PD-1/PD-L1 pathway leads to abnormal activation of $CD8^+$ T cells and infiltration into myocardial tissue, while inhibiting Treg function [49]. Blocking PD-L1/CTLA-4 and PD-1/PD-L1 or lymphocyte-activation gene 3 (LAG-3) has been shown to increase lactate dehydrogenase (LDH) release from cardiac cells. The increased LDH release is a direct marker of myocardial cell membrane integrity disruption and cytotoxic damage, indicating that combination ICI therapy, by breaking down cardiac immune tolerance, results in more severe myocardial cytolysis [9].

If immune tolerance is permanently broken, acute myocarditis can progress to a chronic inflammatory condition associated with sustained cytokine release, infiltration of immune cells, and stromal activation. Collectively, these processes create a profibrotic microenvironment bridging immune dysregulation with fibroblast activation and ECM remodeling, in which TGF-β-centered signaling acts as an essential integrative axis. T cell-mediated myocardial injury similar to that observed in autoimmunity has been reported in other contexts of immune activation, including infection- and vaccine-associated myocarditis, characterized by inflammatory infiltrates rich in $CD8^+$ T lymphocytes and immune effector cell-mediated injury directed at cardiomyocytes; however, whether these mechanisms can be directly extrapolated to ICI-associated cardiotoxicity remains to be fully established [50].

Imbalance of immune cell subpopulations

ICIs usually induce an imbalance of immune cell subpopulations. This imbalance contributes substantially to pathological processes, including autoimmune myocarditis and vasculitis. Clonally activated cytotoxic $CD8^+$ Temra cells and inflammatory macrophages are also expanded in ICI-induced myocarditis, where they reinforce each other in maintaining sustained myocardial infiltration and injury through IFN-γ and CXCL9/CXCL10-CXCR3 signaling [44]. Single-cell sequencing and functional experiments confirmed this, showing that targeting $CD8^+$ T cell exhaustion or blocking IFN-γ signaling significantly reduces the infiltration of toxic T cells and the expansion of inflammatory macrophages to alleviate myocarditis [51]. More importantly, these data suggest that the axis linking $CD8^+$ T cells with macrophages - rather than multiple independent immune pathways - is a dominant driver of myocardial inflammation.

In myocardial tissue of patients with ICI-induced myocarditis, $CD4^+$ T-cell and $CD68^+$ macrophage infiltration is observed, along with C4D-positive staining in necrotic cardiomyocytes, suggesting that T-cell overactivation and complement-associated myocardial injury may jointly participate in the pathological process of ICI myocarditis [52]. ICI-related cardiac injury is also associated with a shift from Treg-mediated tolerance toward Th17-dominant inflammation, reflected by increased IL-17A-producing $CD4^+$ T cells, reduced $FOXP3^+$ Tregs, and an elevated IL-17A/IL-10 ratio, which together favor persistent myocardial inflammation and functional deterioration [53-55].

Macrophage polarization is another convergent mechanism: PD-1 blockade mediates M1-like inflammatory macrophage activation via mitochondrial DNA-cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING), miR-34a/KLF4, and STAT1/NF-κB/NLRP3-related pathways, thereby enhancing cytokine production and myocardial inflammation [56, 57]. Autophagy-dependent NLRP3 degradation and macrophage reprogramming toward an M2-like phenotype may contribute to the resolution of inflammation and counterbalance pro-inflammatory dominance [58, 59].

Autoimmune responses and the generation of anti-myocardial antibodies

ICIs enhance T cell anti-tumor activity by blocking the PD-1/PD-L1 and/or CTLA-4 signaling pathways. However, they may also disrupt cardiac immune tolerance, leading to autoimmune reactions. Anti-myocardial antibodies are closely associated with autoimmune myocardial injury. Studies have shown that combination ICI therapy significantly increases myocardial $CD8^+$ T cell infiltration and cardiac dysfunction. High titers of autoantibodies against cTnI were detected through ELISA, suggesting that ICIs can induce T/B cell-mediated autoimmune responses [60]. Rapamycin has been shown to improve cardiac function by inhibiting the production of cardiac anti-troponin I antibodies, providing mechanistic insights and therapeutic strategies for ICI-induced anti-myocardial antibody-related cardiac toxicity [61].

While autoantibodies, such as anti-cardiac troponin antibodies, have been detected in ICI-associated myocarditis, their pathogenic role remains unclear and may reflect underlying immune activation [62]. Anti-mitochondrial antibodies are more likely a consequence of secondary humoral immune responses following cardiomyocyte injury and antigen release, rather than primary drivers of PD-1 inhibitor-related myocarditis. As immune dysregulation in ICI-induced myocarditis is characterized by increased $CD8^+$ T cell infiltration, a reduction in Tregs, and elevated pro-inflammatory cytokines including IL-6 following PD-1 blockade, these changes highlight the association with enhanced anti-myocardial antibody production [13].

Anti-cardiac myosin (CM) autoantibodies that cross-react with β-adrenergic receptors (βARs) have been identified in myocarditis patients. These antibodies promote myocardial fibrosis through activation of the protein kinase A (PKA) signaling pathway, leading to transcriptional changes and showing a strong association with impaired ventricular function recovery [63]. The major initiating mechanisms of ICI-induced cardiovascular toxicity are summarized in **Table 1**, focusing on key categories of immune dysregulation and representative mechanisms. A summary diagram of the timeline from immune checkpoint blockade and immune dysregu-

Table 1. Initiating mechanisms and key factors of immune dysregulation in ICI-induced cardiovascular toxicity

Mechanistic category	Representative cells or molecules	Core process	Main cardiovascular manifestation	References
Mitochondrial metabolic disorders	$CD8^+$ T cells, macrophages	Oxidative stress-mediated injury	Cardiac dysfunction	[143]
Cytokine storm	IL-6, TNF-α, IFN-γ	Systemic inflammatory activation	Pericardial effusion, vasculitis	[144]
Loss of checkpoint-mediated tolerance	PD-1/PD-L1 pathway	Loss of T-cell inhibition	Fulminant myocarditis	[51]
Treg cell reduction	$FOXP3^+$ Tregs	Loss of immune suppression	Arrhythmia, conduction block	[145]
Pyroptosis	GSDME, GPX4	Inflammatory cell death	Cardiomyocyte necrosis	[43]
NLRP3-dependent inflammasome activation	NLRP3, cysteine-aspartic protease-1 (caspase-1)	Inflammasome signaling	Pericarditis	[146]
Microbiota-driven immune dysregulation	LPS	Systemic inflammation	Atherosclerosis	[147]
Elevated Ang II	AT1R	Vasoconstriction, fibrosis	Hypertension, hypertrophy	[148]
HLA polymorphism	HLA-DRB1	Altered antigen presentation	Myocarditis susceptibility	[149]
TLR4 signaling activation	LPS/TLR4	Inflammatory signaling modulation	Cardiac fibrosis	[84]
TGF-β signaling suppression	Smad7	Impaired fibrotic repair	Ventricular thinning	[150]
CXCR3-dependent T-cell trafficking	$CXCL9/10^+$ macrophages	T-cell recruitment	Myocarditis, dysfunction	[87]
Chemokine-driven leukocyte infiltration	Myofibroblasts, ANGPTL2	Chemokine secretion	Myocarditis, fibrosis	[33]

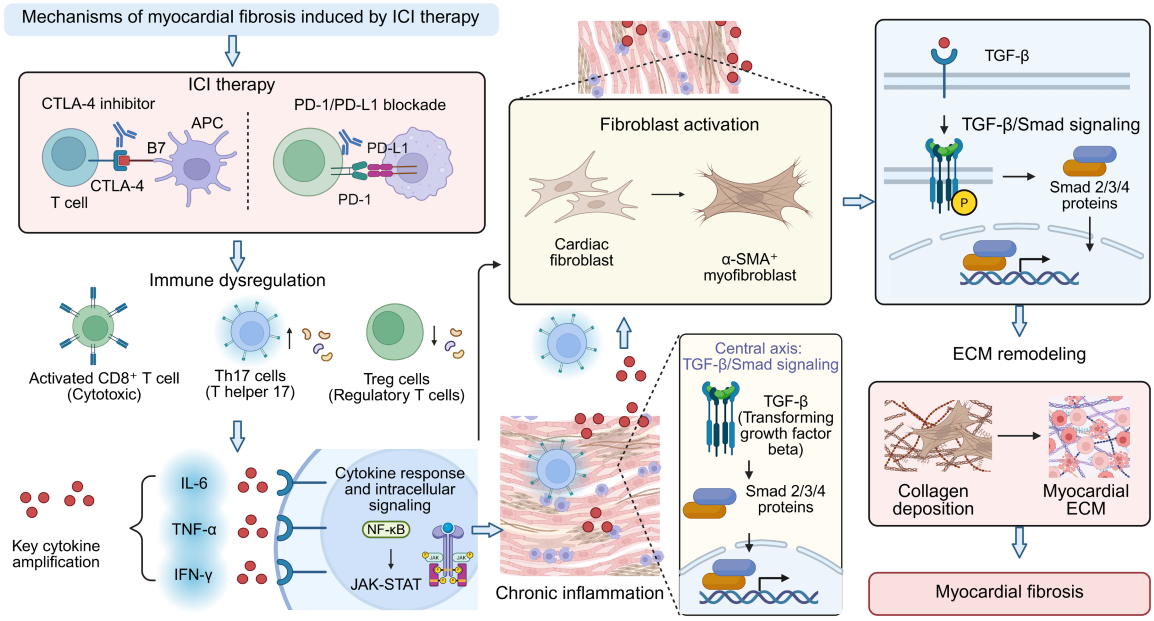

Figure 1. Mechanisms linking immune dysregulation to myocardial fibrosis in ICI-induced cardiovascular toxicity. Immune tolerance is disrupted via blockade of PD-1/PD-L1 and CTLA-4, promoting immune dysregulation characterized by activation of $CD8^+$ T cells, expansion of Th17 cells, and reduction of Treg cells. These alterations contribute to pro-inflammatory cytokine production, including IL-6, TNF-α, and IFN-γ, leading to persistent inflammatory signaling via pathways such as NF-κB and JAK/STAT. Prolonged inflammation stimulates fibroblast activation and differentiation into α-SMA-positive myofibroblasts. TGF-β/Smad signaling is the main profibrotic axis that drives ECM remodeling and collagen deposition, ultimately leading to myocardial fibrosis.

lation to chronic inflammation, fibroblast activation, ECM remodeling, and myocardial fibrosis on the basis of these initiating immune abnormalities is shown in **Figure 1**. In the absence of secondary immune triggers, PD-L1 inhibition monotherapy is not thought to directly induce classic autoimmune myocardial reactions; however, evidence suggests that the cancer milieu may influence cardiac immune tolerance via release of cardiac antigens such as αMHC [64].

Mechanisms of immune dysregulation leading to myocardial fibrosis

Chronic inflammation-induced fibrosis signaling pathways

Myocardial fibrosis, as a downstream pathophysiological consequence of sustained immune activation, arises following dysregulation of immune tolerance and inflammation in the microenvironment. Immune dysregulation drives cardiac fibrosis in ICI-induced cardiovascular toxicity via multiple convergent pathways. Activated fibroblasts are increasingly recognized as key mediators linking immune-mediated inflammation with tissue remodeling and fibrosis [65]. TGF-β/Smad signaling represents a well-supported profibrotic axis connecting myocardial injury to fibroblast activation, collagen deposition, and adverse remodeling, whereas IL-6/JAK/STAT3 and NF-κB pathways are mainly supported by preclinical studies and function as upstream inflammatory amplifiers [66]. In checkpoint blockade, some inflammatory mediators and pyroptosis-related signals have been implicated in modulating TGF-β activity in preclinical models, but these mechanisms are primarily derived from experimental studies, and direct clinical validation in ICI-associated cardiotoxicity is lacking [25, 67, 68]. In contrast to the independent or non-overlapping TGF-β-dependent fibrotic pathways generated by macrophages and fibroblasts, crosstalk between these two pathological cell types (such as TNFSF14/LIGHT signaling) appears to primarily act as an amplifying feedback loop rather than functioning independently [69]. SIRT3 appears to function as an inhibitory modulator of TGF-β/Smad3 signaling, but its relevance in ICI-induced fibrotic sequelae

remains unclear, as most relevant data are from non-ICI experimental models and lack clinical validation thus far [70].

The IL-6/JAK/STAT3 axis is more context-dependent, and compared with TGF-β/Smad signaling, it plays a predominant role in primarily amplifying inflammation-driven fibroblast activation rather than directly initiating fibrosis. Blockade of this axis is mechanistically and potentially therapeutically significant in ICI-associated myocardial injury; nevertheless, current evidence remains limited, and detailed discussion of specific agents such as Tocilizumab is more appropriate in the therapeutic strategy section [71, 72].

Accordingly, it is fitting to regard NF-κB and NLRP3 inflammasome signaling as interlinked inflammatory modules rather than separate pathways, since NF-κB activation generally occurs early and facilitates NLRP3 inflammasome assembly. Instead of being drug-specific observations, these pathways ought to be described as convergent inflammatory nodes [73-75]. NF-κB signaling is particularly context-dependent and not always detrimental. For instance, TRAF2-mediated non-canonical NF-κB activation has been shown to exert cardioprotective effects in various experimental models; however, its involvement in the mechanism of ICI-induced cardiotoxicity remains unclear and lacks clinical validation. Persistent or excessive NF-κB activation, in contrast to adaptive but transient upregulation, appears to promote chronic inflammation-driven fibrosis [76]. While ROCK2 signaling has been implicated in cytoskeletal remodeling in the context of fibrotic progression, much of the evidence supporting its role in ICI-associated cardiotoxicity is derived from experimental studies [77].

Activation and transformation of CF

At this time, following myocardial injury, CFs acquire a myofibroblast-like phenotype through profibrotic signaling pathways, as indicated by alpha-smooth muscle actin (α-SMA) expression, enhanced contractility, latent TGF-β activation, and excessive ECM deposition, thereby contributing to pathological scar formation [78, 79]. This transition can additionally be modulated by angiotensin II (Ang II)-related signaling, including DDR2-integrin β1-mediated type I collagen expression and inflammatory cytokine stimulation within the immune-injury microenvironment [80].

Instead of acting as independent pathways, many of these mechanisms can be classified into broad modules comprising cytoskeletal remodeling, transcriptional regulation, and immune-stromal interaction. For fibroblast-to-myofibroblast differentiation, cytoskeletal remodeling-related mechanisms, including actin polymerization and ROCK signaling, are important; however, evidence suggests the presence of distinct early and late phases during this transition [81]. Transcriptional regulators, including C/EBPβ, function as integrative nodes linking inflammatory signaling with fibroblast activation, rather than acting as independent profibrotic drivers [82]. Fibroblast subsets, such as $CD248^{+}$ cells, primarily reinforce the central TGF-β signaling axis rather than representing independent profibrotic pathways [83].

The participation of innate immune signaling pathways, including lipopolysaccharide (LPS)/Toll-like receptor 4 (TLR4), in inflammatory responses has been observed by demonstrating that HDAC6 inhibition can not only attenuate LPS-induced inflammation through suppression of the TLR4-mitogen-activated protein kinase (MAPK)/NF-κB pathways, but may also indirectly affect fibroblast activation [84]. CFs modulate monocyte recruitment by pro-inflammatory (LPS) or pro-fibrotic (TGF-β1) stimulation. They additionally promote differentiation into M1/M2 macrophages [85]. These include macrophage-fibroblast crosstalk mechanisms, such as IL-18-related signaling, which converge on the TGF-β/mothers against decapentaplegic homolog 2/3 (SMAD2/3) axis and act as amplifiers rather than independent drivers of fibrosis [86]. These studies show that fibroblast-specific deletion of IL-1R1 potently reduces the expression of pro-fibrotic genes, such as Col1a1 and Mmp3, and improves cardiac function, providing hypothesis-generating evidence; however, direct clinical data from ICI-treated patients are still lacking [87]. Thus, overall these findings suggest that fibroblast activation is regulated by a larger hierarchical network, in which TGF-β signaling serves as the central axis, while other pathways function primarily as modulators rather than independent drivers.

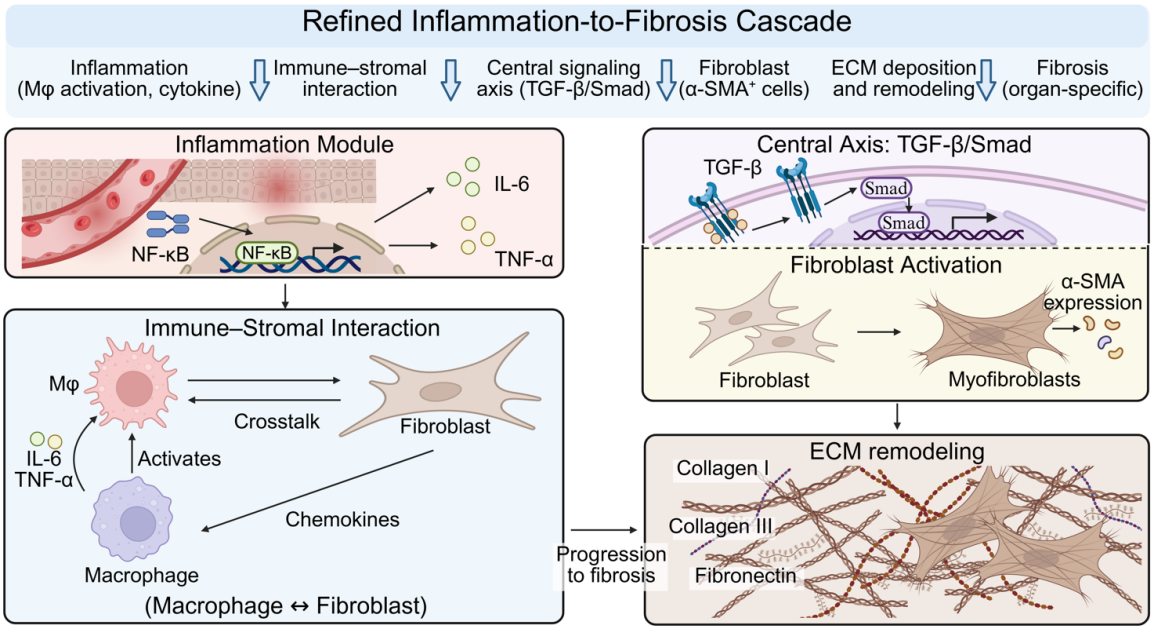

Figure 2. Refined inflammation-to-fibrosis cascade in ICI-induced myocardial injury. Overview of immune-mediated myocardial fibrosis: the schematic summarizes the key modules of immune-mediated myocardial fibrosis. Chronic inflammation activates NF-κB and upstream cytokines (IL-6, TNF-α) involved in immune-stromal cell crosstalk with fibroblasts. Central TGF-β/Smad signaling drives fibroblast-to-myofibroblast differentiation (α-SMA$^+$ cells) and ECM deposition (Collagen I/III, Fibronectin), resulting in organ-specific fibrosis. Other modulatory pathways (IL-6/JAK/STAT3, NLRP3 inflammasome) amplify the profibrotic response without being independent drivers.

ECM metabolic imbalance

Immune-mediated myocardial fibrosis at the tissue level involves ECM remodeling that leads to an imbalance between matrix synthesis and degradation, rather than just collagen accumulation. Fibroblast-specific IL-1R1 signaling mainly regulates ECM turnover through modulation of the matrix metalloproteinase (MMP) system, rather than directly stimulating collagen synthesis [88]. In pressure-overloaded myocardium, activated myofibroblasts inhibit MMP-3/8 expression and stimulate tissue inhibitor of metalloproteinase 1 (TIMP-1) synthesis via a TGF-β/Smad3-dependent pathway to maintain ECM homeostasis. By contrast, in the absence of Smad3 signaling, ECM degradation is increased, leading to the release of collagen-derived pro-inflammatory factors, such as PGP, which initiate macrophage-driven inflammatory responses. As a consequence, the structural integrity of ECM is disrupted, further aggravating myocardial cell damage [3].

Although long-term immunosuppressive therapy can disturb the MMP/TIMP balance and is associated with ECM accumulation, it appears to act as a downstream remodeling mechanism rather than as a primary driver of myocardial fibrosis [89]. In conclusion, zinc deficiency could play a secondary modulatory role that primarily aggravates ECM imbalance via oxidative stress-related mechanisms rather than functioning as an independent fibrotic remodeling driver [90]. The context-dependent matrix remodeling that MMP-7 deficiency illustrates underscores the notion that a given mechanism can improve plaque stability but increase cardiac fibrosis by reducing vascular smooth muscle cell apoptosis [91]. These data show that ECM remodeling is not a simple linear process of collagen accumulation, but instead exhibits a context-dependent balance among matrix degradation, oxidative stress, inflammatory activation, and tissue repair.

Collectively, ECM remodeling should be understood as a dynamic balance regulated by a limited number of core mechanisms, rather than multiple independent signaling pathways, many of which are further modulated at the post-transcriptional level by ncRNAs. **Figure 2** summarizes the major mechanisms linking immune dysregulation to myocardial fibrosis, where-

by sequential progression from chronic inflammation and immune-stromal interaction leads to TGF-β/Smad-dependent fibroblast activation, ECM remodeling, and fibrosis.

The role of ncRNAs in immune dysregulation and myocardial fibrosis

The role of ncRNAs in immune regulation

Inspired by the aforementioned immune and fibrotic pathways, both of which are intricately regulated at the post-transcriptional level, ncRNAs have recently attracted increasing attention as critical mediators connecting immune dysfunction with myocardial fibrosis. Despite the limited evidence for ICI-induced cardiac toxicity, microRNA (miRNA)-mediated regulation of myocardial fibrosis has been demonstrated in non-ICI cardiac injury models. Specifically, miR-208b and miR-21 are known to enhance cardiac fibrotic accumulation by upregulating TGF-β1 and Smad-3 expression, as well as activating the TGF-β1/Smad-3 signaling pathway, supporting the broader involvement of ncRNAs in immune-fibrotic crosstalk [92]. miR-21 is a context-dependent modulator linking immune activation and fibrotic remodeling; nevertheless, prospective validation of its clinical relevance to ICI-induced cardiovascular toxicity is lacking, with the majority of supporting data originating from pre-clinical studies. In immune cells, miR-21 can cause M2-like macrophage polarization by targeting PDCD4 and regulate the phenotypic transition of macrophages while interacting with checkpoint-related pathways such as the PD-1/PD-L1 pathway; under certain conditions, it may play a pro-inflammatory role via upregulation of inflammatory cytokines [93-95]. In fibrotic conditions, miR-21 promotes fibroblast activation and ECM deposition primarily by potentiating TGF-β/SMAD2/3 signaling through inhibition of SMAD7.

miR-29b-3p regulates Th1 cell differentiation by targeting T-bet, thereby integrating immune regulation with fibrotic processes. The miR-29 family has been reported to play an anti-fibrotic role in myocardial fibrosis, mainly through inhibiting the TGF-β/Smad3 signaling pathway and ECM-related gene expression [96, 97]. Long non-coding RNA (lncRNA) H19 can antagonize the anti-fibrotic role of miR-29 by activating the VEGFA/TGF-β axis, thereby facilitating fibroblast proliferation and collagen deposition [98]. In general, these ncRNAs could be broadly classified into pro-fibrotic regulators that strengthen TGF-β signaling, such as miR-21 and H19, and anti-fibrotic regulators, which inhibit ECM deposition, such as the miR-29 family.

The association of ncRNAs with the fibrosis process

According to the above functional classification, most ncRNAs involved in ICI-induced myocardial fibrosis primarily converge on several key pathways, with TGF-β signaling appearing as one of the most clinically relevant regulatory axes, together with ECM remodeling and fibroblast activation. Phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway-related miR-21 promotes ECM deposition and fibroblast activation via upregulation of profibrotic markers [99]. It also enhances myofibroblast differentiation by modulating ERK/TGF-β/Smad and related signaling pathways, indicating a convergent effect on TGF-β-mediated fibrosis [5, 100]. Moreover, miR-21 may regulate fibroblast phenotypic transition through Notch-related signaling, further contributing to myocardial fibrosis.

Other miRNAs also have roles in CF activation through canonical profibrotic pathways, with miR-96-5p and miR-26a being key examples that converge on TGF-β-related signaling networks [101, 102]. Among lncRNAs, MALAT1 is involved mainly in CF proliferation and ECM deposition through TGF-β-related signaling mechanisms [103]. NEAT1 is associated with fibroblast activation and inflammatory responses, at least partly through NLRP3-related signaling pathways, suggesting that ncRNA-mediated regulation integrates fibrotic and inflammatory signaling modules [104].

Several lncRNAs, such as RMST, promote ECM remodeling through lysyl oxidase (LOX)-related pathways and then activate fibroblasts [105]. While a small number of circular RNAs (circRNAs), such as circRNA_000203 and circSMAD4, have been verified to be involved in the process of fibrosis through competing endogenous RNA (ceRNA) networks, most studies rely on in vitro or animal models with limited clinical relevance [106, 107]. CircCAMTA1 and hsa_circ_0004104 are other examples that also regulate fibroblast activation through mechanisms associated with transforming growth

factor-β (TGF-β) receptor-signaling pathways [108, 109]. Thus, the ncRNA-mediated regulatory networks delineated suggest that many lncRNAs and circRNAs converge on a few core profibrotic pathways rather than act as independent drivers of fibrosis. However, the majority of data available at present are from experimental models, and the clinical relevance awaits proper definition due to heterogeneity and insufficient validation. By contrast, axes related to TGF-β signaling and ECM remodeling may represent more stable biomarkers or therapeutic targets. These limitations, at a larger scale, highlight the translational gap between mechanistic discoveries and clinical implementation for ICI-associated cardiovascular toxicity.

Therapeutic targets and intervention strategies

Immune modulation interventions

Immune homeostasis is disrupted, and this reflects ICI-induced cardiovascular toxicity. Interventions should be staged, as this is the time when they can target different pathological stages of the disease through immune modulation. Common strategies employed include cytokine-targeted therapies, Treg cell adoptive transfer, and graded intervention strategies. Clinical studies indicate the potential of Tocilizumab, an IL-6 receptor antagonist, for corticosteroid-refractory PD-1 inhibitor-associated myocarditis with increased serum IL-6 and ongoing inflammatory activity [110, 111]. In addition, in ICI-associated myocarditis, increased IL-6, CXCL9/CXCL10, left ventricular inflammatory uptake, and Th1/Th17 activation all contribute to support the hypothesis that an IL-6-related inflammatory axis could both be a biomarker and theoretically a therapeutic target for refractory disease [112].

Higher-dose corticosteroids with infliximab may be effective in improving cardiac function, but the risk of infection from combined immunosuppressive therapy warrants careful individualization of treatment strategies, emphasizing the importance of a multidisciplinary approach [113]. Baricitinib reduces ICI-related cardiac inflammation and fibrosis via inhibition of the JAK1/STAT3 signaling pathway, through a reduction of pro-inflammatory cytokine release, and promotion of macrophage polarization toward an M2-like phenotype [36]. Targeting members of this signaling circuit, when directed by markers of immune activation such as CD86 receptor occupancy, as well as comprehensive respiratory muscle failure screening, may synergistically tailor therapy with prognostic benefit using systemic immune-modulating agents (Abatacept and Ruxolitinib) in severe ICI myocarditis [114]. Combination therapy with Abatacept, Ruxolitinib, and prednisone has been reported to reverse life-threatening ICI-induced myocarditis via blockade of CD80/CD86-mediated T-cell co-stimulation and suppression of downstream inflammatory signaling [115].

Many experimental modalities that restore Th17/Treg balance or suppress TLR4/NF-κB signaling have shown protective effects in autoimmune myocarditis models. For example, Coriolus versicolor has been demonstrated to reduce pro-inflammatory $CD4^+$ T-cell and $CD68^+$ macrophage infiltration, decrease IL-17 and TNF-α levels, and increase IL-10 expression in experimental autoimmune myocarditis models, suggesting a potential immunomodulatory effect. However, its clinical relevance, particularly in ICI-associated cardiovascular toxicity, remains unproven and should be interpreted with caution [116]. Likewise, T cell immunoreceptor with Ig and ITIM domains (TIGIT)-based approaches have shown the potential to restrain abnormal T-cell activation in experimental models; however, they remain highly experimental, and no clinical evidence currently supports their application in ICI-associated cardiotoxicity.

Tregs are a subset of $CD4^+$ T cells with immunosuppressive functions, and their development and function are regulated by the characteristic transcription factor FOXP3. Removal of specific gut microbiota can suppress the number and function of cardiac Tregs (as indicated by downregulation of FOXP3, IL-10, and TGF-β expression), representing a potential strategy for targeting the gut microbiota-immune modulation axis in ICI-related cardiovascular toxicity [117]. Reducing the proportion of Tregs and enhancing $CD8^+$ T cell activation and pro-inflammatory cytokine (such as IL-6) release can induce cardiomyocyte apoptosis and autophagy. This suggests that Treg cell dysfunction is a key mechanism of PD-1 inhibitor-related myocarditis,

and enhancing Treg function (e.g., through adoptive transfer) can alleviate cardiovascular toxicity induced by ICIs [13].

Anti-fibrotic therapy

Two major pathways involved in ICI-induced myocardial fibrosis include the TGF-β/Smad pathway and connective tissue growth factor (CTGF). Deep-tissue MN-Gal microneedle patches loaded with Galunisertib provide sustained local TGF-β inhibition, thereby suppressing TGF-β/Smad2 signaling, reducing fibroblast activation and myocardial fibrosis, as well as improving ventricular remodeling [118]. In infarcted hearts, hiPSC-derived non-cardiac cells may undergo TGF-β-dependent transdifferentiation into myofibroblast-like cells, whereas Galunisertib inhibits this process by blocking the Smad/Snail/mTOR pathway, resulting in reduced fibrotic remodeling [119].

Pirfenidone enhances matrix remodeling through reducing IL-1β, IL-6, TGF-β, and collagen I/III deposition while increasing elastin expression [120]. Pirfenidone inhibits TGF-β/Smad signaling and attenuates CF proliferation, migration, α-SMA expression, and collagen I/III accumulation in Ang II-induced myocardial fibrosis [121]. Sustained release of Pirfenidone from Pirfenidone-loaded PFP-PFD@NDs-PEG-APM prevents TGF-β/Smad signaling activation, CF-to-myofibroblast transformation, and excess collagen deposition after myocardial infarction in rats [122].

CTGF is a downstream mediator of TGF-β signaling and represents a promising anti-fibrotic therapeutic target. Administration of CTGF monoclonal antibodies has been shown to enhance cardiac repair programs while attenuating the expression of inflammation- and fibrosis-related genes [123]. lncR-30245 promotes myocardial fibroblast proliferation and collagen deposition by inhibiting PPAR-γ and upregulating CTGF, whereas lncR-30245 knockout reduces fibrosis and improves cardiac function [124]. TGF-β1-induced CTGF expression promotes atrial fibroblast activation and collagen deposition, while HGF counteracts this profibrotic process by inhibiting CTGF [125]. CTGF/CCN2 mRNA is also increased in isoproterenol-induced cardiac fibrosis, further supporting CTGF as a shared profibrotic mediator [126].

Combined treatment strategies

The core of combined treatment strategies lies in optimizing the ICI administration regimen and integrating cardiovascular protective drugs for preventive use, thereby reducing the risk of cardiovascular toxicity. Studies have shown that ICI combination therapy (such as ipilimumab + nivolumab) offers impressive clinical benefits in cancer treatment, but significantly increases the risk of cardiac inflammation compared to monotherapy, with a higher mortality rate in combination therapy-associated cardiac inflammation. Therefore, optimizing the ICI treatment plan by using monotherapy with lower doses instead of combination therapy, to reduce the risk of cardiovascular toxicity induced by ICIs, has become a critical issue that clinicians must consider [127].

When undergoing ICI treatment, patients receiving extended dosing intervals (compared to standard intervals) show significantly improved overall survival, suggesting the safety and potential efficacy advantages of optimized dosing schedules, which also help alleviate cardiac toxicity [128]. Studies indicate that after anti-PD-1-based immunotherapy (either monotherapy or in combination with anti-CTLA-4), patients may develop delayed irAEs, necessitating regular toxicity monitoring. The combination of biomarkers (such as troponin, B-type natriuretic peptide [BNP]) and imaging (cardiac MRI) to assess toxicity, followed by timely suspension or dose reduction of ICIs, is recommended [129]. Candidate biomarkers of this kind have been difficult to validate clinically as they are highly variable between patients, lack reproducible results across cohorts, and lack standardized detection platform exists.

The contribution of several mechanisms of action further underlines the potential advantages of combining immune modulation and cardiovascular protection in patients at risk for symptomatic cardiotoxicity. In summary, angiotensin-converting enzyme inhibitor (ACEI) and angiotensin receptor blocker (ARB) can play an important role in myocardial fibrosis by inhibiting the RAAS during ICI treatment. Collagen deposition is modulated by enalapril to reduce myocardial fibrosis and cardiomyocyte hypertrophy [130]. Captopril plus valsartan simultaneously ameliorates left ventricular remodel-

Table 2. Therapeutic targets and intervention strategies for ICI-induced cardiovascular toxicity

Therapeutic Target	Target Type	Mechanism of Action	Intervention Strategy	Therapeutic Effect	Related Experimental Studies	References
T-Cell LAG-3 and Dendritic Cell PD-L1 Expression	Immune Checkpoint Molecule	Cytokine signaling dysregulation; enhanced Treg activity and reduced effector T cell populations	Troponin monitoring; corticosteroid therapy and second-line treatment	Significantly reduced mortality in immune-related myocarditis	Nanostring analysis of myocardial biopsy; immune phenotyping of PBMCs	[151]
Proinflammatory cytokines TNF-α and IL-6	Proinflammatory cytokines	Inhibition of the TNF-α signaling pathway	Infliximab treatment at 5 mg/kg	Comparable MACE-free survival; mortality rate of 23%	Case-control study by Cautela et al	[110]
IFN-γ	Cytokines	IFN-γ-induced expansion of inflammatory macrophages	Blockade of IFN-γ signaling	Amelioration of myocarditis	Antibody neutralization and cell depletion experiments	[152]
NLRP3 inflammasome	Inflammatory signaling complex	Inhibition of NLRP3 activation → reduction of pathogenic immune infiltration	MCC950-mediated inhibition	Improvement of cardiac function/inhibition of tumor growth	ICI-bearing mouse model; single-cell RNA sequencing (immune network analysis); therapeutic intervention with MCC950	[35]
Inflammatory cytokines (IL-6, CXCL9, CXCL10, CXCL13)	Soluble inflammatory mediators and immune cell receptors	Inhibition of hyperactivated T cells and cytokine release	High-dose corticosteroids combined with IVIG	Improving clinical outcomes in patients with early-stage myocarditis	Diagnostic sensitivity study of ^{68}Ga-DOTATOC PET/CT	[112]
CXCR3-CXCL9/10 Axis	Chemokine Receptor-Ligand Axis	Blockade of T Cell Migration and Cardiac Infiltration	Application of CXCR3 Inhibitor	Improved Survival and Attenuated Myocarditis	Murine Genetic Models and Single-Cell Multi-Omics Analysis	[42]
IL-17A	Cytokines	Inhibition of IL-17 Signaling	Anti-IL-17A Antibody Therapy	Prevention of Cardiac Dysfunction	Antibody-Based Intervention in C57BL/6J Mice	[55]
Caspase-1	Proteases	Activation of GSDMD to induce pyroptosis	Blocking pyroptosis by applying the caspase-1 inhibitor Z-YVAD-FMK	Improves cardiac function and attenuates fibrosis	Mouse model of RIHD	[25]
IL-15, IL-4I1, CD4$^+$ TCM	Cytokines, immune factors, and T-cell subsets	Increase CD4$^+$ central memory T (TCM) cells and reduce inflammation	IL-15 combination therapy	Reduce the risk of cardiac fibrosis	Mouse tumor model experiment	[31]
JAK1/STAT3	Kinase signaling pathway	Promote macrophage M2 polarization	Intragastric administration of baricitinib	Improve cardiac function and alleviate inflammatory fibrosis	Mouse model and in vitro macrophage experiments	[36]
MHC-II	Immunoregulatory molecules	Statins indirectly reduce MHC-II synthesis by inhibiting transcriptional regulators	Statins	Cardiac function recovery and successful ICI rechallenge	Immunohistochemical analysis of T-cell subsets	[153]
PD-1, CTLA-4	Immune checkpoint molecules	CD8$^+$ T cells recognize α-myosin, leading to myocardial injury	Anti-CD8 antibody therapy and α-myosin-targeted intervention	CD8-depleting antibody significantly improves mouse survival rate	Pdcd1$^{-/-}$Ctla4$^{+/-}$ mouse model, single-cell RNA/TCR sequencing, and T-cell clonotype analysis of patient samples	[154]
NF-κB	Transcription factors	NF-κB activation and upregulation of inflammatory cytokines	Antibody therapy targeting interleukin-1β and interleukin-6	Cardioprotection	Validation in cell and mouse models	[155]
PD-1, PD-L1, CTLA-4	Immune checkpoint factors	Blockade of immunosuppressive signaling	Discontinuation of ICIs, followed by high-dose corticosteroids and immunosuppressants	Reduced mortality rate	ATRIUM randomized controlled trial	[156]

ing and improves myocardial structure and function by reducing LVEDV, LVESV, and collagen deposition [131]. Losartan reduced myocardial endothelial-to-mesenchymal transition and fibrosis in spontaneously hypertensive rats by inhibiting TGF-β/Smad signaling [132]. EXP3179, as a losartan metabolite, decreased myocardial fibrosis and left ventricular stiffness by inhibiting CTGF/LOX-mediated collagen crosslinking [133]. Still, the prospective clinical validation of ACEI/ARB-based cardiovascular protection in preventing or counteracting ICI-induced myocardial fibrosis remains to be established. The integrated therapeutic strategy holds an actionable clinical translation for the management of ICI-induced cardiovascular toxicity through "immune regimen optimization and cardiovascular drug intervention". **Table 2** summarizes the key molecular targets currently investigated to prevent or mitigate cardiovascular toxicity in patients undergoing ICI therapy, as well as their mechanisms of action and reported preclinical and clinical intervention strategies.

Ongoing challenges and future directions

Unresolved issues

Since the cardiovascular toxicity induced by ICIs also exhibits great interindividual variability, it may be attributed to genetic background factors. Certain human leukocyte antigen (HLA) variations may predispose to organ-specific irAEs by promoting autoimmunity and susceptibility [134]. Still, the direct correlation of HLA with myocarditis or fibrosis needs to be confirmed in large cohort studies. GWAS may identify relevant regulatory roles of these SNPs on T cell activation or inflammatory pathways (such as the IL-2/STAT4 signaling pathway, antigen presentation pathways, and PD-1 gene loci), thus providing genetic predispositions that inform risk assessment and therapeutic strategies [135].

IL-6 receptor inhibitors may effectively block the TGF-β1/Smad3-MMP2/9 signaling pathway to diminish myocardial collagen deposition and fibrosis area, and improve cardiac function, thus acting as a potential option for partial reversal of the fibrotic process in ischemic myocardial remodeling [136]. However, in clinical practice, advanced fibrosis is often accompanied by irreversible myocardial remodeling. One of the major difficulties is assessing the relative weight of factors driving fibrosis and thereby identifying the appropriate treatment window. Future research should combine longitudinal CMR imaging with tissue biopsies to derive molecular thresholds for reversibility.

Translational medicine needs

With current biomarkers, including troponin and BNP, lacking specificity, this underscores the urgent need for additional highly sensitive tools. Circulating free DNA (cfDNA) methylation markers can specifically identify myocardial cell death, with cfDNA exhibiting myocardial cell-specific methylation patterns (e.g., FAM101A loci). This enables non-invasive, quantitative assessment of myocardial cell death, and many studies have confirmed its early detection of myocardial injury in diseases such as acute myocardial infarction and sepsis, correlating with prognosis [137]. In particular, circulating exosomal ncRNAs, such as pro-fibrotic miR-21-5p and anti-fibrotic miR-29c-3p, display dynamic changes associated with the stages of fibrogenesis. For example, PD-1 knockout mice cannot accurately replicate the human immune microenvironment. Short-term perspectives include humanized models, whereby human hematopoietic stem cells and cardiac tissue are transplanted to mimic the cross-immune responses induced by ICIs; and also organoid co-culture systems, where cardiac organoids can be co-cultured with immune cells, which provide a high-throughput/systematic way to test targets for toxicity intervention.

Prospects for personalized treatment

The next steps would depend on advances in toxicity risk stratification models and individualized therapeutic strategies based on multi-omics data, focusing on dynamic monitoring of the cardiac immune microenvironment for precision immunotherapy in personalized treatment. A cardiac toxicity prediction model can be established by combining genomic data (such as HLA genotyping), transcriptomic data (for example, single-cell RNA sequencing), and metabolomic data. This model, together with HLA risk alleles and baseline interleukin-17 levels, would enable in vivo identification of patients at high risk over time. Moreover, individual differences among patients call for more

consolidated and optimized follow-up monitoring experiences by forming focus groups, such as oncological cardiology, as a multi-disciplinary team. Furthermore, assessing the synergistic protective effects of immune-modulating agents or cardiovascular agents will continuously refine clinical approaches to managing immune checkpoint inhibitor-associated cardiac toxicity [138].

Discussion

ICIs have achieved unprecedented breakthroughs in cancer immunotherapy, while their cardiovascular toxicities, especially myocarditis and myocardial fibrosis, have become important challenges for the clinical application of ICIs. ICIs disrupt immune tolerance homeostasis by blocking the PD-1/PD-L1 or CTLA-4 pathways, resulting in excessive T-cell activation and subsequent immune-mediated cardiac injury [139]. This dysregulation of immunity not only promotes the development of myocarditis but also facilitates the progression of myocardial fibrosis; however, the integrative molecular mechanisms linking acute immune activation to chronic fibrotic remodeling remain incompletely understood. Evidence from non-ICI cardiac injury models indicates that myocardial injury can trigger a fibrotic repair program in which myofibroblasts replace damaged tissue with fibrotic scars, while TGF-β activation of Smad-3 promotes myofibroblast proliferation and migration, ultimately contributing to cardiac fibrosis [92].

Contrary to the limited evidence for many other proposed pathways, emerging clinical and translational findings have supported the notion that TGF-β-centered signaling and IL-6-related inflammatory pathways represent a limited subset of mechanisms through which immune dysregulation induced by ICIs drives cardiac fibrosis within a hierarchical network of signaling pathways. Such pathways activate CFs and enhance collagen deposition, resulting in structural damage as well as functional deterioration of myocardial tissue [140]. Recent research on immune regulation and fibrosis by ncRNAs, such as miR-21 and miR-29, has received growing interest, but most current findings are derived from experimental models, and their reproducibility and clinical applicability in ICI-associated cardiovascular toxicity remain uncertain. These molecules modulate immune cell polarization, cytokine secretion, and the expression of fibrosis-related genes, thereby exacerbating ICI-associated cardiovascular toxicity. For example, miR-21 promotes cardiac fibrosis by activating the TGF-β/Smad signaling pathway, whereas miR-29 exerts anti-fibrotic effects by inhibiting the expression of collagen-encoding genes [98]. In addition, the majority of mechanistic insights have been obtained from murine or in vitro model systems that poorly recapitulate the complexity, heterogeneity, and temporal dynamics of the human immune microenvironment exposed to ICIs, thus limiting the translational utility of such discoveries. Immune cell subpopulation imbalances serve as essential mechanistic milestones of ICI-induced cardiovascular toxicity; yet, the specific upstream triggers driving pathogenic $CD8^+$ T-cell activation and Th17 dominance remain poorly defined. Following ICI therapy, excessive activation of $CD8^+$ T cells is a major contributor to myocarditis, while a skewed Th17/Treg ratio accelerates myocardial fibrosis. ICIs enrich $CD8^+$ T-cell and Th17 responses and impair Treg function. This results in immune-mediated cardiomyocyte injury and persistent inflammation [32]. The immune dysfunction further damages cardiomyocytes and induces transdifferentiation of CFs into myofibroblasts, exacerbating myocardial fibrosis.

Although this class of interventions has been explored from the standpoint of clinical management, significant barriers to effective application remain, including the lack of robust and validated biomarkers for early detection, uncertainty regarding the optimal timing and intensity of intervention required, substantial variability in biomarker performance and assay standardization, and a generally limited and heterogeneous evidence base [62]. Furthermore, with the limited sensitivity and specificity of existing diagnostic modalities, and with optimal cut-off values and clinical interpretation not yet determined for most candidate biomarkers, validating these candidate biomarkers to standardize their clinical application remains a major challenge today [141]. Conventional immunosuppressive therapies, such as glucocorticoids, have shown some efficacy in alleviating myocarditis; however, effective treatment options for myocardial fibrosis remain

lacking. Future studies should aim at developing targeted strategies that exploit the immune-fibrosis interplay, as there is emerging evidence of complex crosstalk between immune cells and fibroblasts and potential therapeutic targets for intervention [142]. ICIs induce cardiovascular toxicity through a complicated immune-inflammatory-fibrotic process. Although major advances have been made to dissect the immune dysregulation-fibrosis axis, key knowledge gaps remain, including incomplete mechanistic understanding, limitations of existing experimental models, and barriers to clinical translation and biomarker validation. These issues will need to be addressed in order to improve the prevention and management of ICI-related cardiovascular toxicity.

Disclosure of conflict of interest

None.

Abbreviations

ACEI, angiotensin-converting enzyme inhibitor; AKT, protein kinase B; Ang II, angiotensin II; ANGPTL2, angiopoietin-like protein 2; ARB, angiotensin receptor blocker; BNP, B-type natriuretic peptide; caspase-1, cysteine-aspartic protease-1; ceRNA, competing endogenous RNA; CF, cardiac fibroblast; cfDNA, circulating free DNA; cGAS, cyclic GMP-AMP synthase; circRNAs, circular RNAs; CM, cardiac myosin; CMR, cardiac magnetic resonance; CTGF, connective tissue growth factor; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; cTnI, cardiac troponin I; ECG, electrocardiogram; ECM, extracellular matrix; ERK1/2, extracellular signal-regulated kinase 1/2; FOXP3, forkhead box P3; GSDME, gasdermin E; HLA, human leukocyte antigen; ICIAM, immune checkpoint inhibitor-associated myocarditis; ICIs, immune checkpoint inhibitors; IFN-γ, interferon-gamma; irAEs, immune-related adverse events; JAK, Janus kinase; LAG-3, lymphocyte-activation gene 3; LDH, lactate dehydrogenase; lncRNA, long non-coding RNA; LOX, lysyl oxidase; LPS, lipopolysaccharide; LVD, left ventricular dysfunction; LVEF, left ventricular ejection fraction; MAPK, mitogen-activated protein kinase; miRNA, microRNA; MMP, matrix metalloproteinase; ncRNAs, non-coding RNAs; NF-κB, nuclear factor-kappa B; NLRP3, NOD-like receptor family pyrin domain containing 3; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; Smad, small mothers against decapentaplegic; SMAD2/3, mothers against decapentaplegic homolog 2/3; STAT3, signal transducer and activator of transcription 3; STING, stimulator of interferon genes; TCM, central memory T cells; Temra, T effector memory cells re-expressing CD45RA; TGF-β, transforming growth factor-beta; TIGIT, T cell immunoreceptor with Ig and ITIM domains; TIMP-1, tissue inhibitor of metalloproteinase 1; TLR4, Toll-like receptor 4; TNF-α, tumor necrosis factor-alpha; Treg, regulatory T cells; α-SMA, alpha-smooth muscle actin; βARs, β-adrenergic receptors.

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