

CARDIOVASCULAR DISEASES ASSOCIATED WITH IMMUNE RESPONSE: A COMPREHENSIVE REVIEW

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ABSTRACT

Immune checkpoints naturally help to protect the heart and blood vessels by controlling T-cell activity. They reduce inflammation, promote the development of protective regulatory T-cells and help to maintain stability in blood vessels plaques. When immune checkpoint inhibitor blocks these checkpoints, T-cells can cause inflammation and damage. In some cases, ICIs have also been linked to autoimmune reactions, where the immune system mistakenly attacks heart cells. Normally monoclonal antibodies are the immune checkpoint inhibitors. After escaping from the immune checkpoints, the tumour cells promote T-cell activation which allows them to proliferate. The monoclonal antibodies bind to immune checkpoints and their ligands to prevent inhibition of T-cell activation by tumour cells multiple immune checkpoints inhibitors are developed specially targeted to cytotoxic T-lymphocyte antigen 4 (CTLA 4), programmed death protein 1 (PD-1) and its ligand (PD-L1) and lymphocyte activation gene-3 (LAG-3). This therapy has demonstrated powerful anti-tumour effects and significantly enhanced prognosis for cancer patients. But due to systematic effects, the therapy causes immune related adverse events (irAEs) like atherosclerosis, cardiomyopathy

and myocarditis. These events affect mainly cardiovascular system, liver and lungs and skin. This review highlights the mechanisms through which immune checkpoints protect the cardiovascular system and how blocking them can lead to damage. By understanding this process researchers can identify new ways to prevent or treat cardiovascular irAEs without reducing the effectiveness of cancer treatment. This knowledge could lead to better outcomes for patients by balancing cancer control with good heart health. This simplified overview aims to provide insights into the complex relationship between immune checkpoint inhibitors, immune system regulation and cardiovascular health.

Keywords: *Cardiovascular diseases, CTLA 4, immune response, LAG-3*

Introduction:

Immune checkpoint inhibitors have revolutionized oncotherapy due to their ability to active T cells against cancer cells and protect other cells against damage from cancer. They have secured a crucial place in various cancer treatment. The mechanism of immune checkpoint inhibitors involves the initial recognition and processing of cancer cell antigens by APC (antigen presenting cell) like macrophages, B cells and dendritic cells. These antigens are then presented to T-cells through the MHC (major histocompatibility complex), which initiates the immune response against the cancer cells. There are some crucial secondary co-stimulatory signals which are associated with the interaction between CD80 or CD86 on APCs and CD28 on T cells. The inhibitory molecules such as CTLA-4, PD-1 and its ligand PD-L1 play a crucial role in regulation of T-cell activation and inhibit the excessive immune responses. The function of ICIs is blocking these co-inhibitory molecules, thereby enhancing T-cell mediated anti-tumour responses. Despite their efficiency, ICIs are associated with immune related adverse events (irAEs) affecting various tissues, including thyroid, lungs, colon. The role of PD-1, PD-L1, CTLA-4 pathways has been well documented, but precise mechanism of cardiac irAEs remain unclear. A meta-analysis report states that 8.32% incidence of cardiovascular events among over 21,000 patients who are treated with

ICI in randomized clinical trials (Salem.et. al;2018), (Xavier et.al;2022). irAEs pose a significant threat, ranging from mild arrhythmias to myocarditis, the latter having up to 50% mortality rate. Another meta-analysis states that while cardiovascular irAEs were among the least common (8%), they were the second leading cause of death (25%)(Wang et.al,2018). Upon diagnosis current guidelines recommend temporary or permanent cessation of ICI therapy and immediate immunosuppressive treatment. However, not all patients respond to immunosuppressives and in some critical cases the risk of death may increase by this treatment. In this review we aim to provide a overview about the mechanism of the IC and a possible treatment of cardiac irAEs. (Yousif L. I.et al.2022)

Immune checkpoints:

Immune checkpoint has a regulatory mechanism which regulates the immune response. These checkpoints have a significant role to prevent overactivation of immune cells, which could damage the healthy tissues, it involves proteins such as PD-1, CTLA-4 which are situated on T-cells. These proteins interact with their specific ligands such as PD-L2 TO supress T-cell activity. The T-cell receptor and co stimulatory signals CD28 binds to CD80/ CD86. This binding recruit (PI3R) phosphoinositide 3-kinase which activates the PI3K/Akt pathways. The pathway promotes proliferation differentiation and anti-apoptotic signals in T-cells. After activation the CD8 cells divides into two types of cells cytotoxic T cells and CD cells.

PD-1 is located on the surface of T-cells. It binds only with is two ligands PD-L1 and PD-L2(Ghiotto M.et al.2010). The PD-L1 is situated on hematopoietic cells and tissue cells in different organs. PD-L2 found on macrophages dendritic cells. Binding of PD-L1 and PD-L2 inhibits the T-cell activation through dephosphorylation of P13K-Akt pathway, results low cytokine production, which is associated with inflammation. CTLA- 4 is situated on the surface of activated T-cells CTLA-4 binds to CD80 and CD86 to suppress T-cell activation by down regulating the P13K pathway. In comparison with PD-1, CTLA- 4 inhibits T- cell activation more rapidly(Parry R V.et

al.2005). LAG-3 is expressed on the surface of activated T cells, natural killer cells, B cells and dendritic cells. LAG-3 specially binds to galactin-3 (GAL-3), fibrinogen like protein (FGL-1) and primary ligand MHCII. It gives protection against atherosclerosis by inhibiting excessive T-cell activation.

Immune checkpoint inhibitors in cardiovascular disease and immune response:

Immune checkpoint inhibitors such as pembrolizumab and ipilimumab have transformed cancer treatment by altering immune responses. Pembrolizumab, which inhibits programmed death (PD-L1) and ipilimumab, a blocker of cytotoxic T lymphocyte associated antigen-4, enhance T-cell activity directed against tumours. Though they have endless positive impact on oncology, they may affect the cardiovascular health by altering the immune mediated pathways which causes heart related diseases. Studies of safety records have found possible heart problems from ICIs includes atherosclerosis, cardiomyopathy, myocarditis, heart rhythmic issue (heart block), blood vessel infection (vasculitis), irregular heartbeats, blood clots in veins heart attacks. Pembrolizumab has shown effectiveness against advanced cancers but it carries 1% risk of immune mediated myocarditis which could be fatal if not treated properly. On the other hand, ipilimumab has been associated with cardiac inflammation. Other ICIs like nivolumab (a PD-1 inhibitor) and atezolizumab (targets PD-L1) also have associated cardiac risks. Uses of ICIs can show an arise of irAEs which often require immunosuppressive treatments.

- **Atherosclerosis:** The interaction between PD-1 and PD-L1 suppresses inflammatory T-cell activities and contributes to plaque stability by enhancing Treg activity and decreasing pro-inflammatory cytokines [Vuong J T. et al.2022] Inhibition or removing PD-1/PD-L1 results in heightening inflammation, enlarged plaques which leads to adverse vascular damage. The PD-L1 on endothelial cells defence against the immune related damage by the T-cell function and lower the chances of apoptosis in endothelial cells. CTLA-4 plays a important role in regulating T-cell activation and inflammation, thereby decreasing plaques size and slowing

or lowering the risk of atherosclerosis (Poles K.et al.2020). In the absence of CTLA-4, lesions increase in size which increase the risk of deposition of fatty material on the inner wall of heart. LAG-3 supports the survival of endothelial cells through MAPK /Erk and PI3K/ Akt / mTOR signalling pathways (Hemon P.et al.2011).

- **Myocarditis:** Myocarditis is the inflammation of the heart muscle and is frequently associated with immune checkpoint inhibitor therapies. Although it is uncommon, it carries a high risk of mortality(50% mortality) especially in severe cases (Salem J E.et al.2018). Factors that increase the risk include the specific type of ICI used (anti PD-1/PD-L1 is more risky than anti CTLA-4), combination therapies and radiotherapy, chemotherapy. The breakdown of immune tolerance causes T-cell activation, resulting myocarditis. The PD-L1 expression on endothelial cells offers protective immune separation, which disrupt by ICI treatment. ICIs stimulates T-cells and inflammatory cytokines which increases cardiac inflammation.

- **Cardiomyopathy:** it is a disease in which heart loses its ability to pump blood effectively leads to heart failure arrhythmias and other complications. PD-1 and PDL1 are proteins that help to regulate the immune system and prevent excessive immune responses (Kushnareva E.et al.2022). In mice, lacking PD-1, researchers observed dilated cardiomyopathy (DCM), a condition where the heart becomes enlarge and weak. This was linked to an autoantibodies(immune proteins attacking the body's own heart proteins) but not to immune cell infiltration, suggesting information and altered heartcell behaviour as the Cause (Tay W T.et al.2020). In humans, PD-L1 was found in damaged heart tissues, suggesting it helps to protect the heart by reducing immune activity and inflammation. A genetic variation in the CTLA-4 gene, which is involved in immune regulation, was found more often in DCM patients, hinting that autoimmunity plays a role in the disease, though this variation didn't affect disease progression after one year

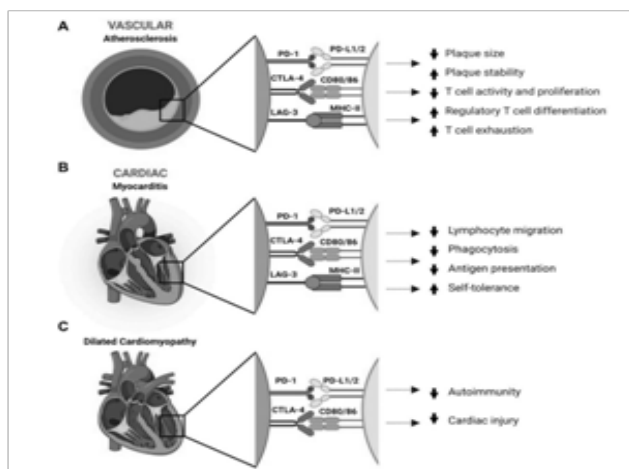


Figure: the role of immune checkpoints PD-1, CTLA-4 and LAG3 in mitigating atherosclerosis, dilated cardiomyopathy, and myocarditis

PATHWAY SPECIFIC THERAPEUTIC TARGETS:

Exploring alternatives to immunosuppressive drugs for managing cardiovascular immune-related adverse events (irAEs) linked to immune checkpoint inhibitors presents possible therapeutic targets in downstream pathways (Matzen E. et al. 2021). Although corticosteroids are currently the first line treatment, there is a growing need for additional therapies due to the diverse patient responses and the potential for tumour progression during recovery. The JAK/STAT pathway which is affected by PD-L1/PD-L2 and CD80/CD86 signalling, is a key focus of research (Kubo S. et al. 2014). JAK inhibitors such as Tofacitinib, have demonstrated potential in managing ICI-induced myocarditis allowing for rapid patient recovery without significant side effects. Nonetheless, caution is warranted as JAK/STAT3 inhibition may promote tumour growth (Henderson Berg M. H. et al. 2022). Additionally, pathways like MAPK/Erk/PI3K/Akt/mTOR are being investigated. Inhibiting mTOR in conjunction with anti PD-L1 therapy has shown advantages in maintaining anti-tumour

efficacy while minimising toxicity (Esfahani k.et al.2019)At the same time MNK1/2 might lower oncogenic activity but could also elicit pro-inflammatory cytokines.Finally targeting the tryptophan pathway influenced by CD80/86 signalling, especially through the enzyme indoleamine 2,3-dioxygenase 1and (IDO1) represents another potential strategy for cancer immunotherapy (Tang k.et al.2021). Current clinical trials involving IDO inhibitors are showing potential across various cancers, although challenges persist, particularly following a failed phase III trial involving pembrolizumab and IDO inhibitors.

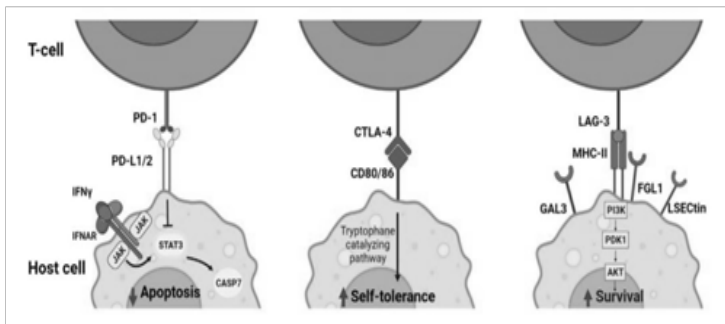


Figure: downstream signalling and effect of immune checkpoint ligands

Perspective:PD-1, PD-L1 and LAG-3 are presently recognised as therapeutic targets in the treatment of cancer. However, approximately 10% of patients receiving immune checkpoint inhibitors (ICIs) experience potentially life threatening cardiovascular immune related adverse events (irAEs) (Xavier et. al.,2022). With the increasing number of ICI being authorised and utilised in clinical settings, the occurrence of cardiovascular irAEs is anticipated to rise. Immune checkpoints down regulate the immune response by influencing various pathways involved in T cell activation, differentiation, and survival, thus diminishing inflammation and fostering tolerance. Moreover, the ligands for immune checkpoints have been found to possess additional intracellular signalling mechanisms that decrease

apoptosis and encourage self-tolerance in target cells such as endothelial cells and cardiomyocytes. The precise mechanisms responsible for the 3 diseases induced by ICIs- atherosclerosis, myocarditis and cardiomyopathy are not fully understood common necessitating further research. Presently, the management of irAEs with corticosteroids does not yield benefits for every patient, and there is an urgent need for downstream targets. There have been recorded cases of successful treatments for ICI-induced myocarditis such as abatacept and randomised trials are in progress. Furthermore, additional research concentrating on the downstream pathways of immune checkpoint ligand host cells, including the JAK/STAT, MAP/Erk/mTOR, PI3K/Akt pathways and IDO1, as potential therapeutic targets is essential. While it is crucial to comprehend the role of human checkpoints in the cardiovascular system, monitoring and prevention are equally significant in clinical practice. It is recommended to conduct serial monitoring using eco-cardiography (ECG) electrocardiograms and biomarkers (like troponin) for patients undergoing immunotherapy, chemotherapy and radiotherapy (Banfill et. al., 2021). According to the latest all patients treated with ICIs should have an ECG and troponin levels assessed baseline with extra ECG evaluation for those with elevated baseline troponin levels (Lyon et al., 2022). In instances of newly occurring abnormalities in ECG readings, changes in biomarkers, or cardiac symptoms at any stage during ICI treatment the guidelines recommend prompt cardio oncology assessment, including additional ECG for evaluating left ventricular ejection fraction and strain analysis. Cardiac magnetic resonance imaging is advised when myocarditis is suspected.

Conclusion:

Immune checkpoint inhibitor therapies have offered successful treatment options and enhance prognosis for a significant number of cancer patients. However, their interaction with the cardiovascular system can be harmful. The blockade of immune checkpoint ligand interactions has been shown to hasten the onset and progression of atherosclerosis heighten inflammation and lead to myocardial infiltration, which can

result in life threatening myocarditis.

Additionally, it is suggested that these therapies made trigger autoimmunity and cause electrophysiological changes in cardiomyocytes, resulting in cardiomyopathy. Understanding the mechanisms related to immune checkpoint and cardiovascular immune related adverse events is crucial to :

- 1) identify preexisting risk factor for improved patient selection
- 2) explore treatment option for cardiovascular irAEs that ideally maintain prolonged anti-tumour ICI effectiveness and
- 3) highlight potential new targets for cancer treatment.

References:

Banfill, K., Giuliani, M., Aznar, M., Franks, K., McWilliam, A., Schmitt, M., Sun, F., Vozenin, M. C., Faivre Finn, C., & IASLC Advanced Radiation Technology committee (2021). Cardiac Toxicity of Thoracic Radiotherapy: Existing Evidence and Future Directions. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer*, 16(2), 216–227. <https://doi.org/10.1016/j.jtho.2020.11.002>

Esfahani, K., Al-Aubodah, T. A., Thebault, P., Lapointe, R., Hudson, M., Johnson, N. A., Baran, D., Bhulaiga, N., Takano, T., Cailhier, J. F., Piccirillo, C. A., & Miller, W. H. (2019). Targeting the mTOR pathway uncouples the efficacy and toxicity of PD-1 blockade in renal transplantation. *Nature communications*, 10(1), 4712. <https://doi.org/10.1038/s41467-019-12628-1>

Ghiotto, M., Gauthier, L., Serriari, N., Pastor, S., Truneh, A., Nunès, J. A., & Olive, D. (2010). PD-L1 and PD-L2 differ in their molecular mechanisms of interaction with PD-1. *International immunology*, 22(8), 651–660. <https://doi.org/10.1093/intimm/dxq049>

Gotsman, I., & Lichtman, A. H. (2007). Targeting interferon-gamma to treat atherosclerosis. *Circulation research*, 101(4), 333–334. <https://doi.org/10.1161/CIRCRESAHA.107.155838>

Hemon, P., Jean-Louis, F., Ramgolam, K., Brignone, C., Viguier, M., Bachelez, H., Triebel, F., Charron, D., Aoudjit, F., Al-Daccak, R., & Michel, L. (2011). MHC class II engagement by

its ligand LAG-3 (CD223) contributes to melanoma resistance to apoptosis. *Journal of immunology* (Baltimore, Md. : 1950), 186(9), 5173–5183. <https://doi.org/10.4049/jimmunol.1002050>

Henderson Berg, M. H., Del Rincón, S. V., & Miller, W. H. (2022). Potential therapies for immune-related adverse events associated with immune checkpoint inhibition: from monoclonal antibodies to kinase inhibition. *Journal for immunotherapy of cancer*, 10(1), e003551. <https://doi.org/10.1136/jitc-2021-003551>

Kubo, S., Yamaoka, K., Kondo, M., Yamagata, K., Zhao, J., Iwata, S., & Tanaka, Y. (2014). The JAK inhibitor, tofacitinib, reduces the T cell stimulatory capacity of human monocyte-derived dendritic cells. *Annals of the rheumatic diseases*, 73(12), 2192–2198. <https://doi.org/10.1136/annrheumdis-2013-203756>

Kushnareva, E., Kushnarev, V., Artemyeva, A., Mitrofanova, L., & Moiseeva, O. (2022). Myocardial PD-L1 Expression in Patients With Ischemic and Non-ischemic Heart Failure. *Frontiers in cardiovascular medicine*, 8, 759972. <https://doi.org/10.3389/fcvm.2021.759972>

Lyon, A. R., López-Fernández, T., Couch, L. S., Asteggiano, R., Aznar, M. C., Bergler-Klein, J., Boriani, G., Cardinale, D., Cordoba, R., Cosyns, B., Cutter, D. J., de Azambuja, E., de Boer, R. A., Dent, S. F., Farmakis, D., Gevaert, S. A., Gorog, D. A., Herrmann, J., Lenihan, D., Moslehi, J., ... ESC Scientific Document Group (2022). 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). *European heart journal*, 43(41), 4229–4361. <https://doi.org/10.1093/eurheartj/ehac244>

Matzen, E., Bartels, L. E., Løgstrup, B., Horskaer, S., Stilling, C., & Donskov, F. (2021). Immune checkpoint inhibitor-induced myocarditis in cancer patients: a case report and review of reported cases. *Cardio-oncology* (London, England), 7(1), 27. <https://doi.org/10.1186/s40959-021-00114-x>

Parry, R. V., Chemnitz, J. M., Frauwirth, K. A., Lanfranco, A. R., Braunstein, I., Kobayashi, S. V., Linsley, P. S., Thompson, C. B., & Riley, J. L. (2005). CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. *Molecular and*

cellular biology, 25(21), 9543–9553. <https://doi.org/10.1128/MCB.25.21.9543-9553.2005>

Salem, J. E., Manouchehri, A., Moey, M., Lebrun-Vignes, B., Bastarache, L., Pariente, A., Gobert, A., Spano, J. P., Balko, J. M., Bonaca, M. P., Roden, D. M., Johnson, D. B., & Moslehi, J. J. (2018). Cardiovascular toxicities associated with immune checkpoint inhibitors: an observational, retrospective, pharmacovigilance study. *The Lancet. Oncology*, 19(12), 1579–1589. [https://doi.org/10.1016/S1470-2045\(18\)30608-9](https://doi.org/10.1016/S1470-2045(18)30608-9)

Tang, K., Wu, Y. H., Song, Y., & Yu, B. (2021). Indoleamine 2,3-dioxygenase 1 (IDO1) inhibitors in clinical trials for cancer immunotherapy. *Journal of hematology & oncology*, 14(1), 68. <https://doi.org/10.1186/s13045-021-01080-8>

Tay, W. T., Fang, Y. H., Beh, S. T., Liu, Y. W., Hsu, L. W., Yen, C. J., & Liu, P. Y. (2020). Programmed Cell Death-1: Programmed Cell Death-Ligand 1 Interaction Protects Human Cardiomyocytes Against T-Cell Mediated Inflammation and Apoptosis Response In Vitro. *International journal of molecular sciences*, 21(7), 2399. <https://doi.org/10.3390/ijms21072399>

Vuong, J. T., Stein-Merlob, A. F., Nayeri, A., Sallam, T., Neilan, T. G., & Yang, E. H. (2022). Immune Checkpoint Therapies and Atherosclerosis: Mechanisms and Clinical Implications: JACC State-of-the-Art Review. *Journal of the American College of Cardiology*, 79(6), 577–593. <https://doi.org/10.1016/j.jacc.2021.11.048>

Wang, J., Sanmamed, M. F., Datar, I., Su, T. T., Ji, L., Sun, J., Chen, L., Chen, Y., Zhu, G., Yin, W., Zheng, L., Zhou, T., Badri, T., Yao, S., Zhu, S., Boto, A., Sznol, M., Melero, I., Vignali, D. A. A., Schalper, K., ... Chen, L. (2019). Fibrinogen-like Protein 1 Is a Major Immune Inhibitory Ligand of LAG-3. *Cell*, 176(1-2), 334–347.e12. <https://doi.org/10.1016/j.cell.2018.11.010>

Xavier, C. B., Lopes, C. D. H., Harada, G., Peres, E. D. B., Katz, A., & Jardim, D. L. (2022). Cardiovascular toxicity following immune checkpoint inhibitors: A systematic review and meta-analysis. *Translational oncology*, 19, 101383. <https://doi.org/10.1016/j.tranon.2022.101383>

Yousif, L. I., Tanja, A. A., de Boer, R. A., Teske, A. J., & Meijers, W. C. (2022). The role of immune checkpoints in cardiovascular disease. *Frontiers in pharmacology*, 13, 989431. <https://doi.org/10.3389/fphar.2022.989431>